EXHIBIT 3

United States Patent [19]

Haruki et al.

[11] **3,932,264**

[45] **Jan. 13, 1976**

[54] **ELECTROPHORETIC MEASUREMENT SYSTEM INCLUDING MEANS FOR DETERMINING ZONE BOUNDRIES**

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[52] U.S. Cl. ..... **204/299**; 204/180 G; 204/180 R; 204/195

[51] Int. Cl.² **B01K 5/00**

[58] Field of Search ..... 204/180 R, 180 G, 299, 204/195

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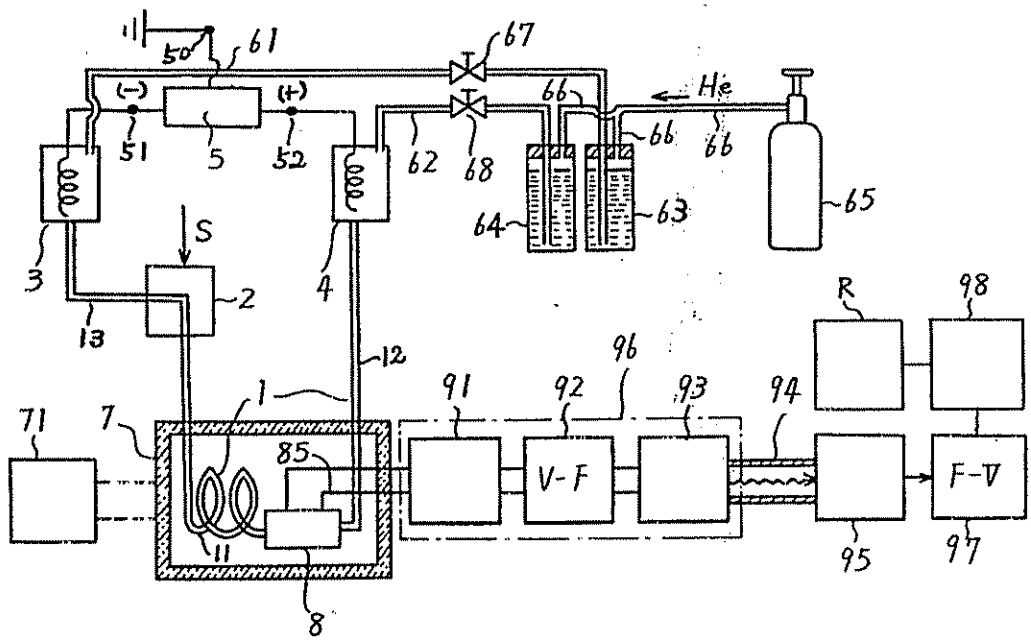
Primary Examiner—John H. Mack
Assistant Examiner—A. C. Prescott
Attorney, Agent, or Firm—George B. Oujevolk

[57] **ABSTRACT**

An electrophoretic measurement system for isotachophoresis includes a potential gradient detector, an impedance converter, a voltage to frequency converter, a signal transmission system composed of, for example, electromagnetic wave transmitter means and its receiver spaced therefrom, frequency to voltage converter means, in series connection. This arrangement facilitates insulation of the sensing electrodes of the detector from the ground and prevents a formation of bubbles and deposits on the electrodes, so that the electrophoresis processes are performed under stable condition and high resolution and high sensitivity are obtained. Besides a higher migration current can be used to shorten analysis time.

14 Claims, 15 Drawing Figures



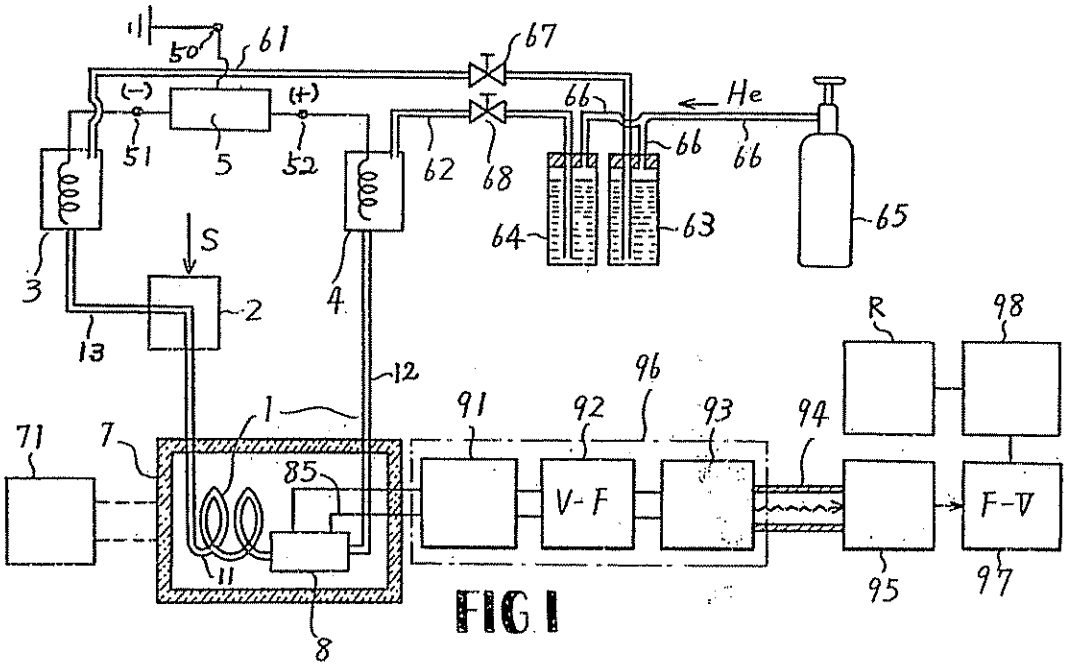


FIG 1

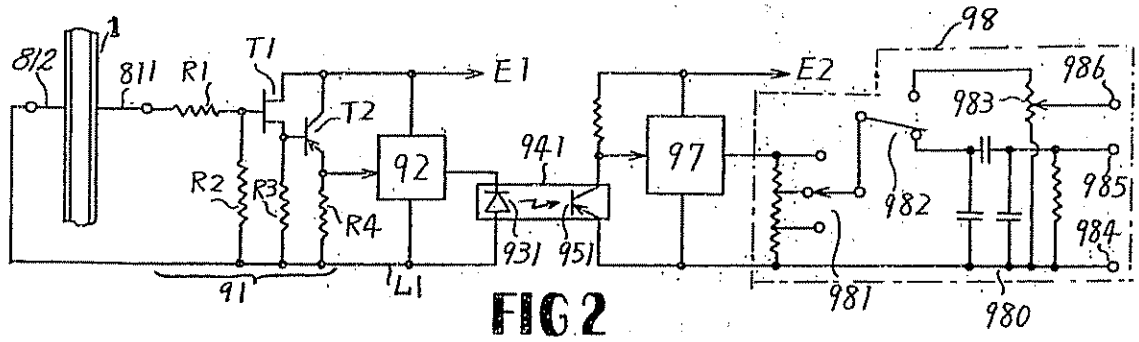


FIG 2

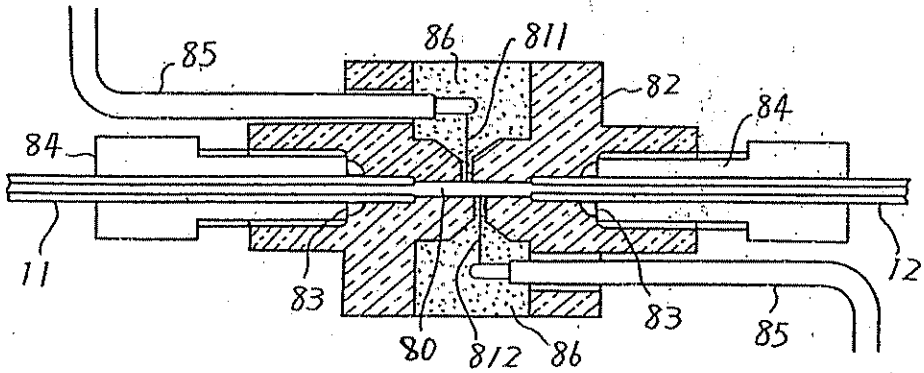


FIG 3

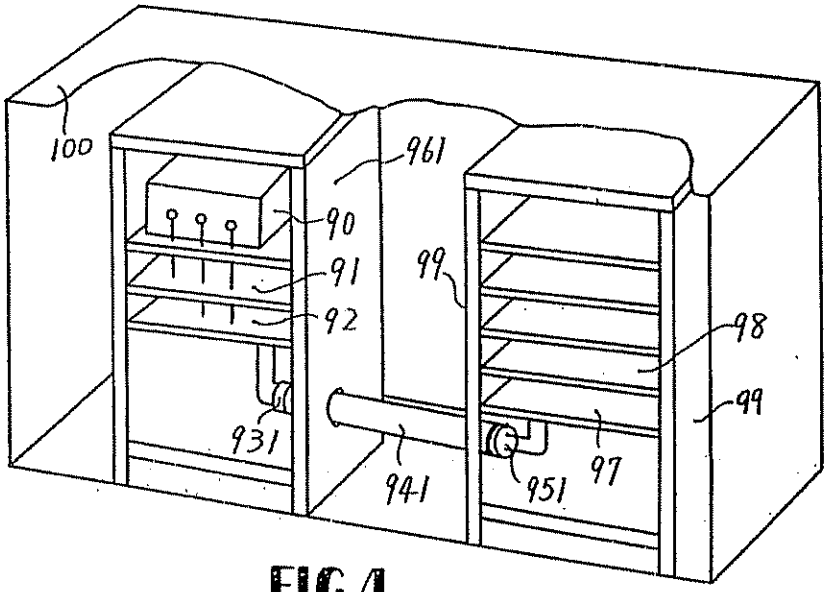


FIG. 4

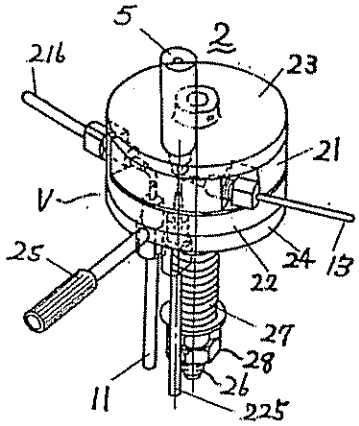


FIG. 5B

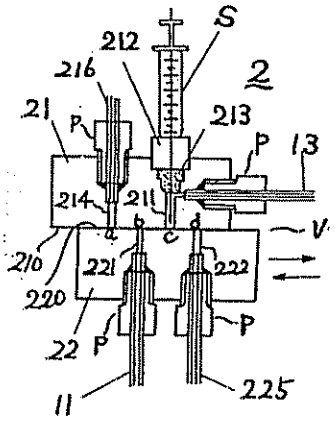


FIG. 5A

FIG.6A

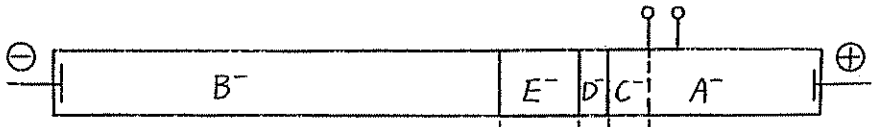


FIG.6B

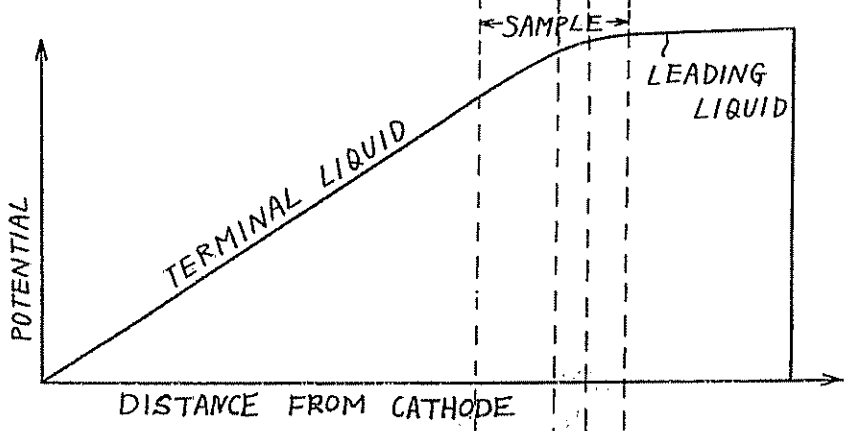


FIG.6C

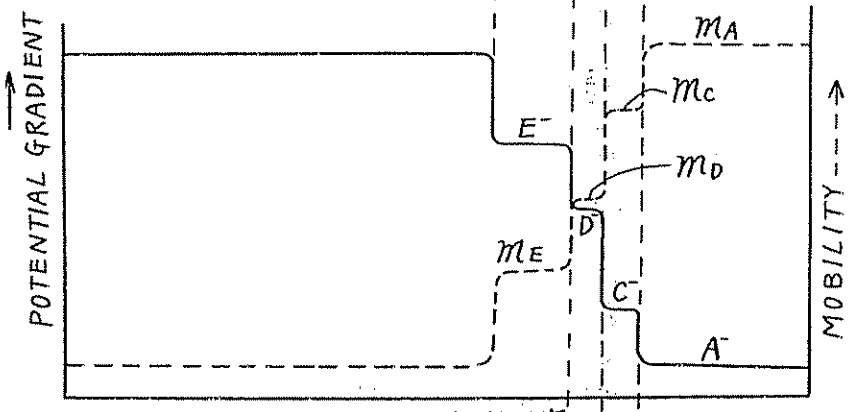
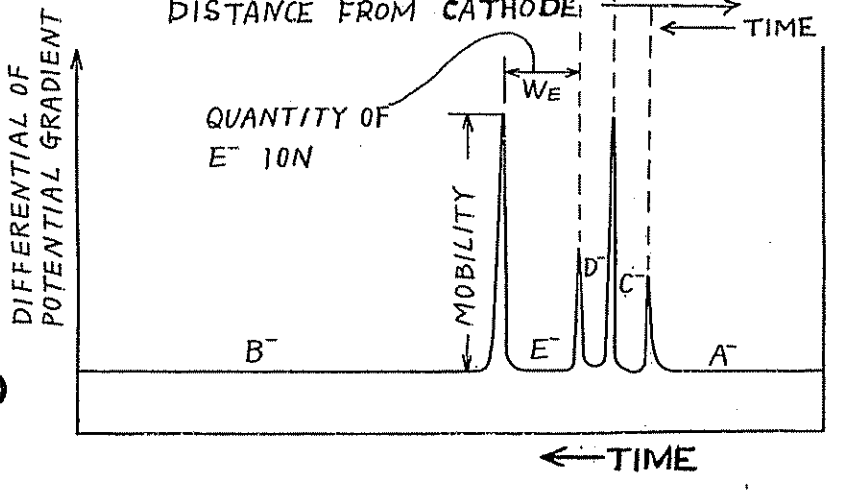


FIG.6D



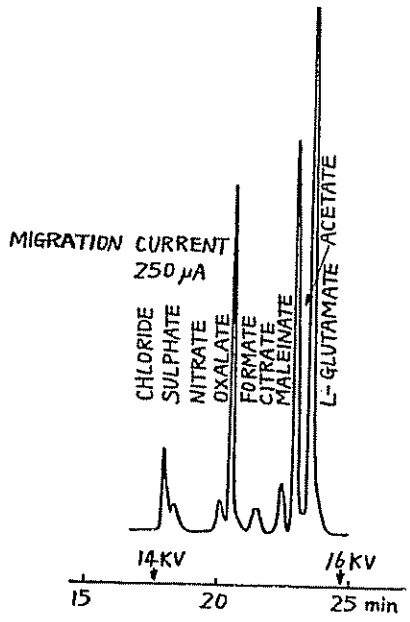


FIG. 7B

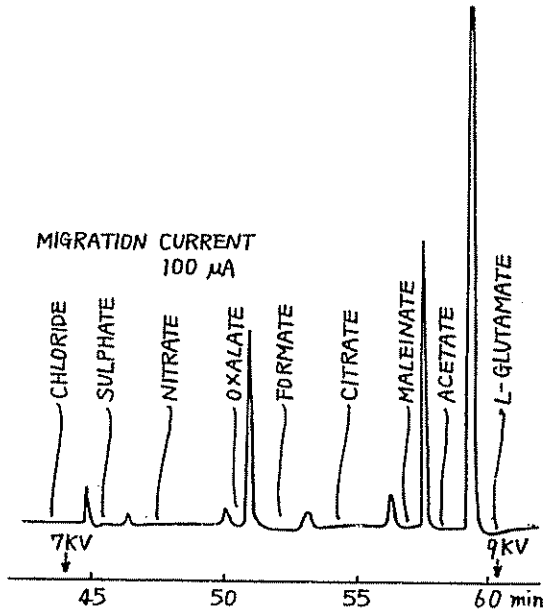


FIG. 7A

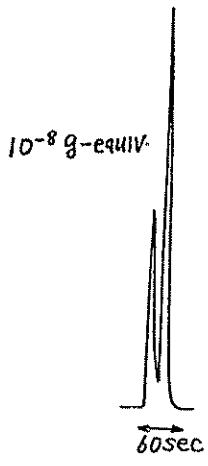


FIG. 8A

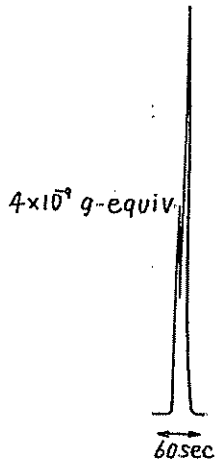


FIG. 8B

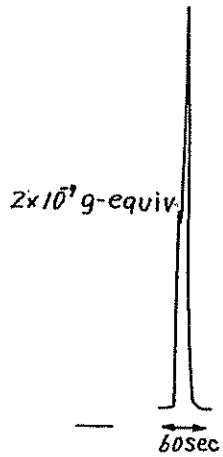


FIG. 8C

ELECTROPHORETIC MEASUREMENT SYSTEM INCLUDING MEANS FOR DETERMINING ZONE BOUNDRIES

BACKGROUND OF THE INVENTION

The present invention relates to an electrophoretic measurement system, and more particularly to a new system for measuring zone boundaries in an isotachophoresis system.

BRIEF DESCRIPTION OF THE PRIOR ART

In isotachophoresis, a potential difference is applied across a capillary tube containing a leading electrolyte and a terminal electrolyte holding a sample solution which is generally interposed at or near the boundary of the electrolytes. The sample ions are e.g. anions, the leading electrolyte contains a single sort of anions and their mobility is higher than that of sample ions; the terminal electrolyte also contains a single sort of anions but with a lower mobility than that of sample ions. Then the anions within the sample move towards the anode between the anions of the leading electrolyte and the anions of the terminal electrolyte.

The unknown anions in the sample will be separated slowly into distinct layers in the order of their mobilities. The boundaries formed between each layer are detected by a detector.

Heretofore various detectors for capillary-type isotachophoretic system have been proposed, but none of them has been put to practical use.

The most important reason for this may be the fact that there has not been developed a method that can detect zone boundaries in a capillary tube with a high resolution for most types of samples. A thermometric detector has not been found satisfactory due to its rather low resolution. An ultra-violet detector has not been used as a universal detector because of the existence of many compounds that do not absorb UV. A potential gradient detector and a conductivity detector have also been developed as promising high resolution detectors. These detectors, however, have not yet been put to practical use, because electrochemical bubbles and deposits produced on the sensing electrodes tend to impede the smooth performance of isotachophoresis.

OBJECTS OF THE INVENTION

Accordingly, the general object of the invention is to provide an improved electrophoresis analyzer which overcomes to a high degree many of the disadvantage of the prior art.

Another object of the invention is to provide an improved electrophoresis analyzer with stable operation without the formation of bubbles and deposits on the sensing electrodes.

A further object of the invention is to provide an improved electrophoresis analyzer having a good resolution and sensitivity.

A still further object of the invention is to provide an improved electrophoresis analyzer capable of shortening the time for analysis.

These and other objects, features and advantages of the present invention will become more apparent upon a reading of the following detailed specification and drawings, wherein:

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic representation of an electrophoresis analyzer system according to the present invention, in which some portions of it is shown in section, with a circuit diagram;

FIG. 2 represents a preferred embodiment of a measuring circuit of zone boundaries.

FIG. 3 shows a sectional view of a potential gradient detector preferably used in the present invention;

FIG. 4 is a perspective view of one embodiment of the arrangement of a measuring circuit.

FIG. 5A and FIG. 5B show a partly schematic, vertical, sectional view of a sampling valve for introducing a sample between the leading and the terminal electrolytes and a perspective view of a practical embodiment.

FIG. 6A to FIG. 6D illustrate the operation of the electrophoresis process in the present invention.

FIGS. 7A, 7B, FIG. 8A to FIG. 8C illustrate some electropherograms obtained with use of an analyzer of the invention.

DETAILED DESCRIPTION

In accordance with the present invention, high resolution high sensitivity and shortening of time are achieved in an electrophoresis analyzer, by preventing bubble formation on the sensing electrodes.

Referring first to FIG. 1. There is shown a capillary tube 1, which is composed of three portions 11, 12 and 13, for an isotachophoretic column with an injection system 2. The tube 1 is connected to the current stabilized high voltage power supply 5 for providing an adjustable constant direct current, through a leading electrode compartment 3 and a terminal electrode compartment 4.

The power supply 5 has a transformer, a centre tap 50 with an out coil which is grounded, and polarity of the output can be reversed.

The compartments 3 and 4 are respectively connected to a first tube 61 and a second tube 62 which are deeply inserted into a leading electrolyte tank 63 and a terminal electrolyte tank 64. The tops of each of the tanks 63 and 64 are connected to a pressurized source of inert gas 65 e.g. He by tubing 66. Tubes 61 and 62 are provided with stop valves 67 and 68 for controling the liquid flow for the compartment 3 and 4 from the tank 63 and 64.

The tubes, 1, 61, 62 and 66, the port or the sample injection system 2, the compartments 3, 4, the tanks 63, 64 and valves 67, 68 are composed of a chemically stable insulating materials such as PTFE (Trade Mark: Teflon).

The column tube 1 has, for example, 100 cm length, 0.5 mm inner diameter and 2 mm outer diameter; the greatest part of the capillary tube 1 is placed in a bath 7 having a thermostat with a regulating power supply 71.

The system for measuring of zone boundaries is preferably composed of: a sensing cell 8, which is preferably formed as a gradient detector hereinafter described, situated near the end of the capillary tube 1; an impedance converter 91 with ultra-high input impedance and lower output impedance, connected to the output terminal of the sensing cell 8 by a PTFE sleeved wire 85 through the wall of the bath 7 with thermostat; a voltage to frequency converter 92 for generating a pulse signal whose frequency is proportional to the output voltage from the converter 91; signal transmis-

sion system T for electromagnetic or ultrasonic waves which is preferably composed of a radiation or luminescent source 93 such as photo-diode for generating an optical signal in response to the output signal of the converter 92, an optical transmitting channel 94 and a photo-electric converter 95 such as a photo-transister or phototube for generating an electrical signal in response to the light signal from the source 93; insulating means 96 for isolating the potential of the circuit 91, 92 from ground to maintain the potential of the circuit 91, 92, 93 nearly equal to that of the sensing cell 8, for preventing any current leakage between each sensing electrode and the ground; a frequency to voltage converter 97 for generating the electrical signal proportional to the frequency of the output pulse from the sensor 95; a signal processing circuit 98 including a differential circuit for generating the signal representing the zone boundaries of separated ions; a recorder R having a constant chart speed for recording a electropherogram.

Also the aforementioned signal transmission system may be some other systems capable of insulating a detector from a recorder, and converters 92, 97 may be a frequency modulator and its demodulator.

Referring to FIG. 2, the output terminals of a pair of sensing electrode 811, 812 are connected to the gate terminal of a field effect transistor T1 through an input resistor R1 of ultra high resistance, for example 10^9 – 10^{10} ohms and to the line L1 (not grounded) respectively. The gate of the transistor T1 and line L1 are connected by a second ultra high resistance resistor R2. A source terminal of the transistor T1 is connected to the line L1 by a resistor R3, and to the base terminal of the transistor T2 with an emitter-follower connection. The emitter of the transistor T2 is connected to the voltage-frequency converter 92 used for energizing a diode 931. A power supply E1 for the circuit 91 and 92 is preferably composed of an insulated transformer having very little leakage current or an electric cell or a battery. The aforesaid signal transmission system is composed of an optical radiation source of a diode 931, an opaque resin tube 941 and a phototransistor 951.

Besides the signal transmission system may be composed of, for example, radio wave or infrared, ultraviolet, radient rays transmitter means and their receivers.

A circuit 98 is for example composed of a range selector 981, a switch for selecting a potentiometer 983 or a differentiator 980.

Referring to FIG. 3, the sensing cell 8 comprises mainly a capillary channel 80 formed between the capillary tubes 11 and 12 inserted tightly into a methacrylic resin block member 82 and a pair of sensing electrodes 811, 812 of Pt wire threaded tightly through the block normal to the axis of the channel 80, wherein both electrodes are separated by a small distance along the channel.

There are seals 83 of PTFE seal tapes between the tubes 11, 12 and the block 82; the fitting plugs 84 screwed in the block 82 press the seals 83 and support the tube 11 and 12. The other ends of the electrodes 811, 812 are soldered to each of the bare ends of the cable wires 85 threaded through the block 82 and fixed with Araldite (Trade Mark of CIBA) resin 86.

It is preferable that the diameter of the channel 80 is somewhat larger than the inner diameter of the tube 11, 12 and the electrodes 811, 812 are formed as thin as possible (for example: 0.08 mm of diameter) and the ends of the electrode 811, 812 are sized to just agree with the wall of the channel 80; so that there is a minimum chance of discharging ions moving in the sensing cell 8, so that bubble formation at the electrodes can be suppressed to some extent.

In FIG. 4 there is shown an embodiment of the arrangement of the measuring circuit, wherein a case 961 of high insulation material such as methacrylic resin contains the impedance maching circuit 91, the voltage to frequency converter 92 and a power supply 90 for the circuit 91, 92. A power supply 90 is preferably composed of an electric cell or a battery, which is convenient for isolating the circuit 92 and 92 from the ground without a special insulated transformer.

The output terminals of the circuit 92 are connected to a photodiode 931 mounted in one end of the opaque tube 941 of high insulation material. The tube 941 is extended through the case 91 and a second case 99 containing the circuit 97, 98 etc. In the other end of the tube 941 there is mounted a photocell or a phototransistor 951, the output terminals of which are connected to the input terminals of the frequency to voltage converter 97. Numerals 984, 985, 986 are the output terminals of the circuit 98 to the recorder R.

Referring to FIG. 5A and 5B there are shown embodiments of a sample injection system 2 with a sampling valve V suitable for introducing a sample just on the boundary surface between the leading and the terminal electrolytes.

The valve as shown in FIG. 5A is simplified for explanation of operation and comprises a pair of valve members formed as a disc-shaped stationary PTFE valve member 21 and a disc-shaped movable PTFE valve member 22 which have smooth plain contact surfaces 210 and 220.

The valve member 22 has a channel 221 connected to a capillary tube 11 and a channel 222 connected to a first drain tank (not shown) with a tubing 225.

The valve member 21 has a first channel 211 communicating with a syringe needle guide 212 through a septum 213 and a second channel 214. The channel 214 is connected to a second drain tank (not shown) with a tubing 216. The first channel 211 is further connected to a tubing 11 to the leading electrode compartment 3. Besides, the valve is so constituted that it has three positions of valve operation; a first position for filling the capillary tubes 11, 12 and 13 with a leading electrolyte and a terminal electrolyte, a port *a* to coincide with a port *b* and a port *c* to coincide with a port *d*; in the second position, which is shown in FIG. 5A, for preliminary injection of a sample, there is no communication among the ports *a*, *b*, *c*, and *d*; in the third position port *b* and port *c* communicate each other, for introducing a sample on the boundary surface of the both electrolytes. For these purposes, a line *ab* and a line *cd* are arranged parallel to a direction of sliding of a movable valve member 22 and a length of *ab* is equal to that of *cb*.

Fig. 5B shows the embodiment of sampling valve 2 which is convenient for practical use, which comprises a stationary valve member 21 mounted to a stationary steel disk 23 and a rotatory valve member 22 mounted to a rotating steel disc 24. A disc 24 has a rotation handle 25, a shaft 26, a pressurizing spring 27 placed between the steel disc 24 and a nut 28. The general constructions of the valve and operation is substantially the same as the embodiment of FIG. 5A.

In operation prior to measuring, the leading electrolyte and terminal electrolyte are charged in the capil-

lary tube 1, then a sample is introduced between the both electrolytes.

In the first step, a movable disc 22 of the sampling valve V is placed in the first position wherein the port *a*, *c* communicate with the ports *b*, *d* respectively, and a valve 67 and a valve 68 are opened, then a terminal electrolyte in the tank 64 flows through a tubing 62, compartment 4, second portion 12 of a capillary tube 1, a sensing cell 8, a first portion 11 of the tube 1 and the port *b*, *a* of the valve V and a tubing 216 to a drain tank; a leading electrolyte in the tank 63 flows through a tubing 61, a leading electrode compartment 3, a thrid portion 13 of the capillary tube 1, the ports *c* and *d* and a tubing 225 to the drain tank.

In the second step the valve is operated to the second position as shown in FIG. 5A, and a sample in the mircoliter syringe S is injected gently into the channel 211 through a syringe needle inserted into the channel 211 from the septum 23 to the vicinity of a port *c*, then the sample injected located the channel adjacent to the port *c* with the result that the terminal electrolyte in the tube 13 is slightly pushed back for the leading electrolyte tank 64, to give place to an injected sample.

Thus a boundary is formed between the terminal electrolyte and the sample solution.

In the third step, the valve is operated to the third position wherein a port *b* coincides with a port *c*, so that the leading electrolyte comes in contact with the sample solution. Thus the sample introduction is completed just on the boundary between the leading and the terminal electrolytes.

The tanks 63, 64 then are cut off from the capillary tube 1 by the stop valves 67 and 68, in order to prevent electroendomosis in the capillary tube.

In the migration process, the power supply is put on to start the migration in the capillary tube in the constant temperature bath 7. In the course of migration, for example, each ion with a negative charge migrates for the leading electrode with positive potential according to its mobility, and ions in the sample solution are separated into a series of layer deposited in order of their mobilities.

In the final aspect, as shown in FIG. 6A, 6B, 6C where separation is finished, each zone contains a single sort of ions respectively and migrate at a same speed and a (concentration) density of ions in any zone, for example of the C^- ions, is given by the following equation (1).

$$Ca/Cc = (1 + Mr/Mc)/(1 + Mr/Ma) \quad (1)$$

Where

Ca: (concentration) density of leading ions

Cc: (concentration) density of C^- ions in the zone

Mr: mobility of positive ions

The equation teaches that a density of ions in each completely separated zone has a value independent of the numbers of ions in each zone.

Thus the width of each zone of same ions is proportional to the numbers of ions. FIG. 6D shows an electropherogram recorded as a differential of the output from the potential gradient detector 8. Namely in FIG. 1 a gradient detector 8 generates an output voltage proportional to the potential difference between both sensing electrodes 811, and 812, which is introduced to the voltage to frequency converter 92 through the impedance converter 91.

The converter 93 receiving a pulse signal from the converter 92 generates a radiant energy signal, for example, optical radiation of a diode 931 of the same frequency as the output from the converter 92. The converter 95 (a photocell 951) generates an electric signal in response to the signal transmitting on the path 94 and supplies it to a frequency to voltage converter 97. Thus the converter 97 regenerates an electric signal proportional to the output voltage of the detector 8, without electric potential coupling between them.

The output of the converter 97 is supplied to the recorder R through a range selector 981, a switch 982 or either a differentiating circuit 980 or a potentiometer 983. Thus the recorder writes either a potential gradient curve or a differential curve of the output in a wide range.

Also circuit 98 may be varied so as to enable the recorder to record both curves at the same time.

EXAMPLE I

In the embodiment of FIG. 1, the terminal electrode compartment 3 and the injection port 2 were connected with PTFE tube (inner diameter: 1 mm, outer diameter: 3 mm, length: 10 cm), the injection port 2 and the leading electrolyte compartment 4 were connected with a PTFE capillary tube of 0.5 mm inner diameter, 2 mm outer diameter and 1 mm length.

Then an aqueous solution of 0.01M histidine and 0.01M histidine HC1 as the leading electrolye, and an aqueous solution of 0.01M L-glutamic acid as the terminal electrolyte are charged in the tank 63 and 64 respectively.

Then 5 μl mixture of 0.01M sodium sulphate, 0.01M sodium nitrate, 0.01M sodium oxalate, 0.01M sodium formate, 0.01M sodium citrate, 0.01M sodium maleinate, 0.01M sodium acetate was sampled in a microliter syringe.

Then the sample was introduced just on the boundary surface of the both electrolyte, as hereinbefore described, then migration was performed with migration current of 250 μA.

A temperature of the bath 7 was kept at 20°C during the migration procedures. It was found that no bubbles were generated on the sensing electrodes even at 250 uA migration current. The result obtain through a differential circuit 980 in given in FIG. 7B, which shows that the boundary were detected with sufficient stability.

EXAMPLE II

FIG. 7A shows the electrophoregram obtained with the same sample and same operating condition as that of Example I, except that the migration current is 100 μA and the attenuation rate by a range selecter 981 is half of Example I.

This electropherogram shows a good resolution of the detector. In detail FIG. 7A shows that the zone boundary between sulphate and nitrate having mobility difference of about 10 percent and diffusion coefficient of 10^5 cm^2/sec. can be detected as a peak of 180 uV height, with a noise level of 10 uV, but without any discernible baseline drift. If the peak several times as high as the level of the noise is assumed to be the detection limit, this potential gradient detector can be said to detect the boundary between two zones having a mobility difference of about 1 percent, in case of samples having diffusion coefficient of 10^5 cm^2/sec.

EXAMPLE III

Several experiments were performed to test the separation to trace quantity of samples. Migration was done

for several quantities of samples with 100 μA of migration current. The thermo-bath temperature was kept at 20°C.

FIGS. 8A, 8B and 8C illustrate the electropherograms recorded on the recorder R through the differential circuit 980, for the adipic acid of 10^{-8} gram equivalent, 4×10^{-9} gram equivalent and 2×10^{-9} gram equivalent, using the solution of 0.01M histidine and 0.01M histidine HCl as a leading electrolyte, and the solution of 0.01M glutamic acid as a terminal electrolyte. These results shows the minimum sample size to detect two adjacent boundaries, as peaks, is approximately 2×10^{-9} gram equivalent.

EXAMPLE IV

After about 200 hours' operation at 100 μA of migration current, at 20°C of the bath temperature with the same sample as in FIG. 7A, 7B, the sensitivity and the noise level remained at the almost same level as the electropherograms in FIG. 7A, 7B.

As shown hereinbefore this invention has various advantages, for instance.

1. Through isolation of the sensing cell, which is situated at a high voltage to ground, from the ground potential, by converting the electrical signal provided by the sensing cell into an optical signal, reduction of the current between the sensing electrodes and ground is attained.
2. Through scraping off the projecting parts of the sensing electrodes, reduction of the electric current between the two sensing electrodes is further attained.
3. Through expanding the cross-sectional area of the cell, reduction of the current density in the sensing cell is further attained.
4. As a result of each abovementioned improvement of their co-operation, generation of bubbles in the sensing cell was suppressed or prevented and it is possible to perform stable measurement of zone boundaries up to a high migration current.
5. As a result of (4), good resolution and sensitivity can be obtained.
6. As a result of (4), migration process can be finished in a shorter time.

Although the invention has been described in its preferred form with a certain degree of particulality, it is understood that the present disclosure of the preferred form may be changed in details of construction and the combination and arrangement of parts may be resorted to without departing from the spirit and scope of the invention as hereafter claimed.

What we claim is:

1. An electrophoretic measurement system comprising: an electrophoretic column means (**11, 12, 13**); sample introducing means for introducing a sample into said column means, power means (**5**) for generating a potential gradient along said column means; detector means (**8**) for sensing zone boundaries produced within said column means and providing an electrical output signal; signal transmitter means (**93**) for receiving the output electrical signal from said detector means and transmit it as a wave; converter means (**95**) disposed for receiving said wave and generating an electrical signal in response to said transmitted wave; reading means for reading the output signal from said converter means.

2. The system of claim **1**, wherein said transmitter means comprises a source of optical radiation and an optical path and said converter means comprises a photoelectric sensor.

3. The system of claim **2**, wherein said source of optical radiation is composed of a semiconductor diode.

4. The system of claim **1**, wherein said detector means is a gradient detector for sensing a potential gradient within liquid in said column tube.

5. The system of claim **4**, wherein said detector means comprises a channel with a wall, which communicates to said column means and electrodes disposed apart from each other.

6. The system of claim **4**, wherein said detector electrodes comprises a pair of thin electrodes plugged, in the wall of said channel, without projecting into the inside from the inner surface of the wall.

7. The system of claim **4**, further including a detector output terminal and a transmitter input terminal, an impedance converter, which has a high input impedance and a lower output impedance, interposed between the output terminal of the detector means and the input terminal of said transmitter means.

8. The system of claim **4**, further including: means for generating a pulse signal of the frequency related to the output from said detector means to pulsate the input signal to said transmitter means; means for generating an electrical signal interposed between said converter means and said reading means.

9. An electrophoretic measurement system comprising in combination

a. a tube (**1**) for defining an isotachophoretic column therein said tube (**1**) having central, leading and terminal portions (**11, 12, 13**);
b. at least first and second tanks (**63, 64**) for holding leading and terminal electrolytes connected to said leading and terminal portions;
c. power supply means connected to said tube disposed to apply a current across said central portion to carry out electrophoretic separation therein;
d. a bath tank for holding said tube central portion;
e. valve means (**2**) connected to said tube (**1**) having first and second positions allowing selective coupling to said leading and terminal electrolytes and a third position allowing feeding of a sample to said tube (**1**);
f. sensing means connected to said central portion including a capillary channel (**80**) and first and second sensing electrodes (**811, 812**) disposed on said column means, and electrical units measuring circuitry fed by said sensing electrodes;
g. transmitter means coupled to said sensing means converting the output therefrom to a wave signal;
h. converter means disposed to receive said wave signal and convert said wave signal to an electrical signal; and,
i. reading means coupled to said converter means for displaying the output therefrom.

10. The system of claim **9**, wherein said transmitter means comprises a source of optical radiation and an optical path and said converter means comprises a photoelectric sensor.

11. The system of claim **10**, wherein said source of optical radiation is composed of a semiconductor diode.

12. The system of claim **9** wherein said valve means includes a stationary disc (**21**) and a rotating disc (**22**)

said discs having smooth contact surfaces and being in contact one with the other, first and second channels (211,214) on said stationary disc, first and second channels (221, 222) on said movable disc, and connecting tubing connecting certain channels to said first and second tands (63, 64) and tube portions (11, 12, 13), sample injection means in one of said discs and means to turn said rotatable disc with respect to said stationary disc so that said first and second channels in one disc may separately be disposed opposite one, the other or neither of said first and second channels in the other disc so that a sample may be injected through said sample injection means between a leading and terminal electrolyte fed to said defined isotachophoretic column by said valve means across said channels.

13. The system of claim **1**, wherein the signal transmission line from the output terminals of said detector means to said signal transmitter means is insulated from the ground.

14. The system of claim **1**, wherein the signal differentiating means is interposed between said converter means and said reading means.

* * * * *

EXHIBIT 4

United States Patent [19]

Akiyama

[11] **3,941,678**

[45] **Mar. 2, 1976**

[54] **APPARATUS FOR ELECTROPHORETIC ANALYSIS**

[75] Inventor: **Junichi Akiyama**, Kyoto, Japan

[73] Assignee: **Shimadzu Seisakusho Ltd.**, Kyoto, Japan

[22] Filed: **Feb. 20, 1975**

[21] Appl. No.: **551,275**

[30] **Foreign Application Priority Data**

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Mar. 7, 1974 Japan 49-26961

[52] **U.S. Cl.** ..... **204/299 R**; 204/180 R; 204/180 G

[51] **Int. Cl.²** **B01K 5/00**

[58] **Field of Search** ..... 204/180 R, 180 G, 299

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Primary Examiner—John H. Mack

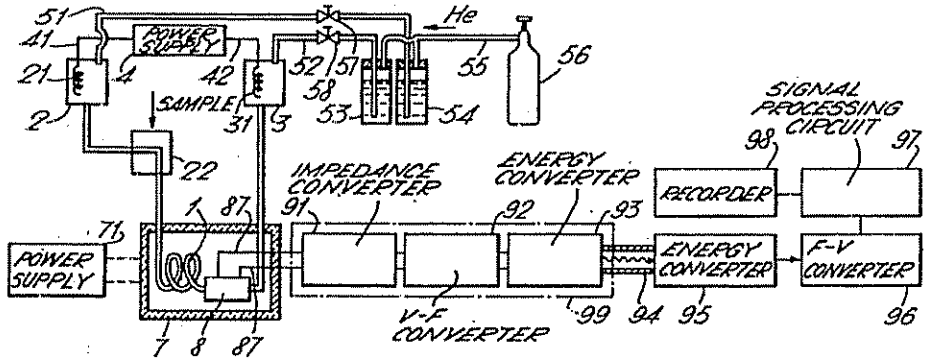
Assistant Examiner—A. C. Prescott

Attorney, Agent, or Firm—Fidelman, Wolffe & Leitner

[57] **ABSTRACT**

Apparatus for electrophoretic analysis of ions or like electrically charged particles, wherein the potential gradients of the different kinds of ions contained in a liquid column formed in an electrophoretic capillary tube are detected and the ratio between potential gradient of a selected one of said different kinds of ions and that of each of the other kinds of ions is calculated to obtain a value relating to the mobility of each of said different kinds of ions.

7 Claims, 6 Drawing Figures



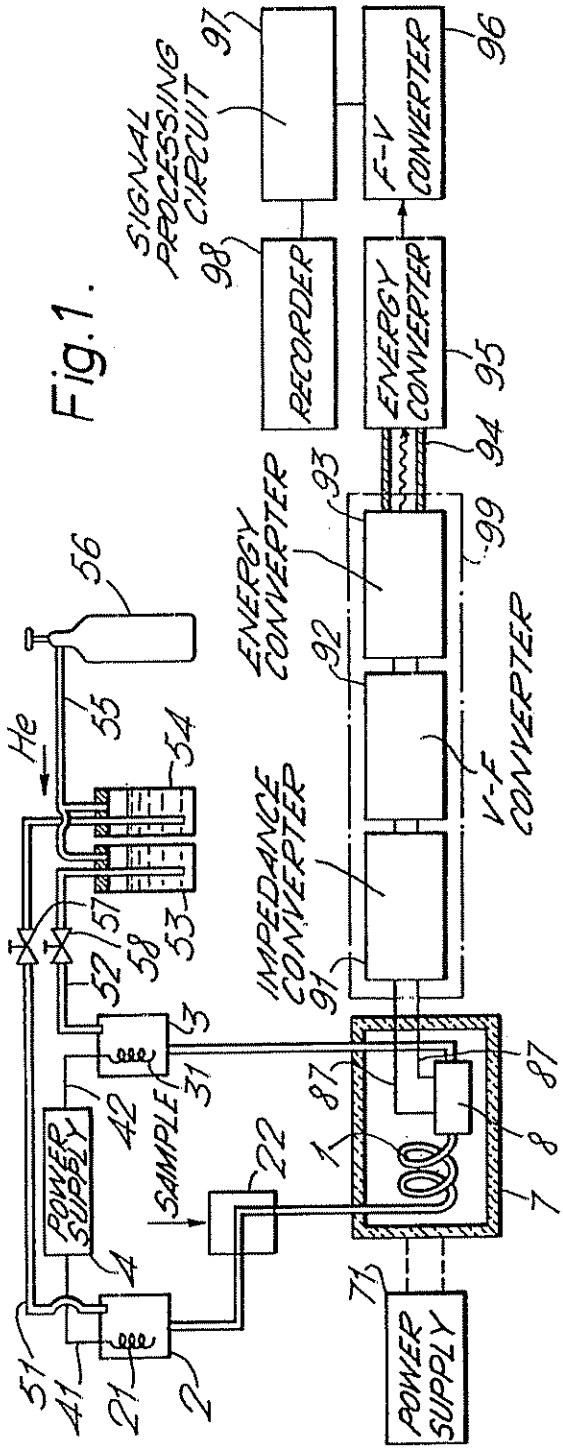

SIGNAL PROCESSING CIRCUIT
97
F-V CONVERTER
96
RECORDER
98
ENERGY CONVERTER
95
94
ENERGY CONVERTER
93
99
V-F CONVERTER
92
IMPEDANCE CONVERTER
91
56
He
55
54
57
58
53
52
3
31
42
POWER SUPPLY
4
51
41
SAMPLE
22
1
87
8
7
71
POWER SUPPLY
21
2

Fig. 1.

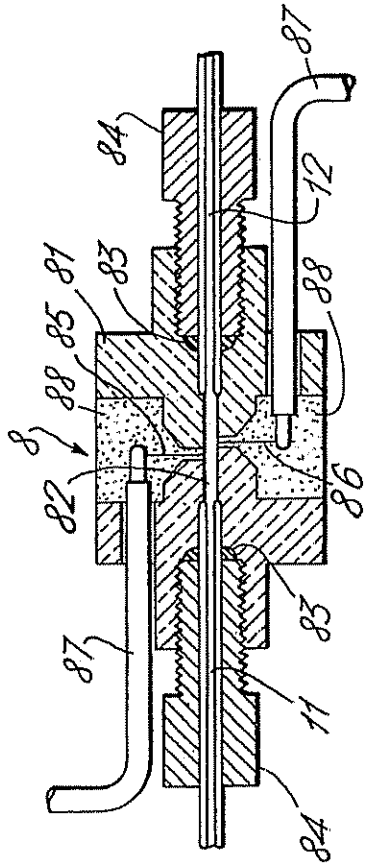

87
84
12
81
83
88
85
88
8
82
86
87
83
11
84

Fig. 2.

Fig.3.

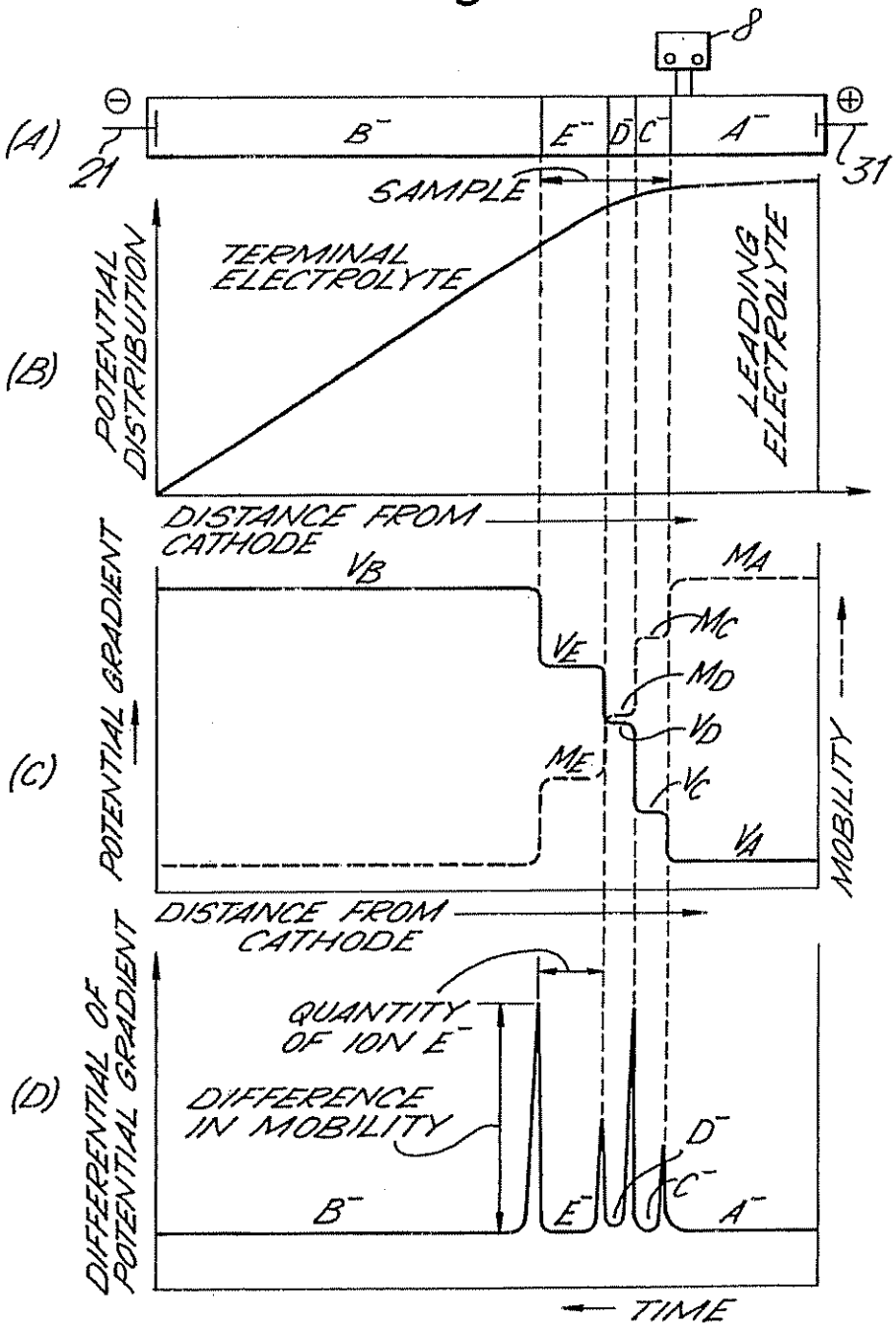

8
(A)
⊖
⊕
B⁻
E⁻
D⁻
C⁻
A⁻
21
31
SAMPLE
(B)
POTENTIAL DISTRIBUTION
TERMINAL ELECTROLYTE
LEADING ELECTROLYTE
DISTANCE FROM CATHODE
(C)
POTENTIAL GRADIENT
MOBILITY
VB
VE
VD
VC
VA
MA
MC
MD
ME
DISTANCE FROM CATHODE
(D)
DIFFERENTIAL OF POTENTIAL GRADIENT
QUANTITY OF ION E⁻
DIFFERENCE IN MOBILITY
B⁻
E⁻
D⁻
C⁻
A⁻
TIME

Fig.4.

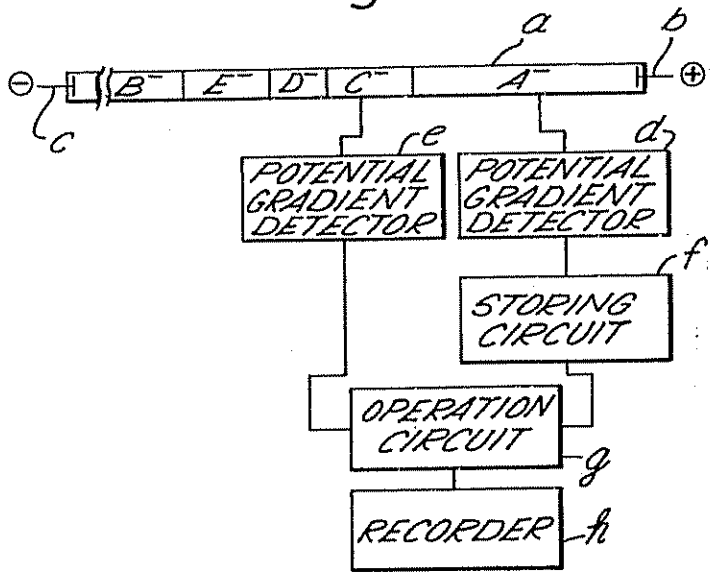

a
b
B⁻
E⁻
D⁻
C⁻
A⁻
c
e
d
POTENTIAL GRADIENT DETECTOR
POTENTIAL GRADIENT DETECTOR
f
STORING CIRCUIT
OPERATION CIRCUIT
g
RECORDER
h

Fig.5.

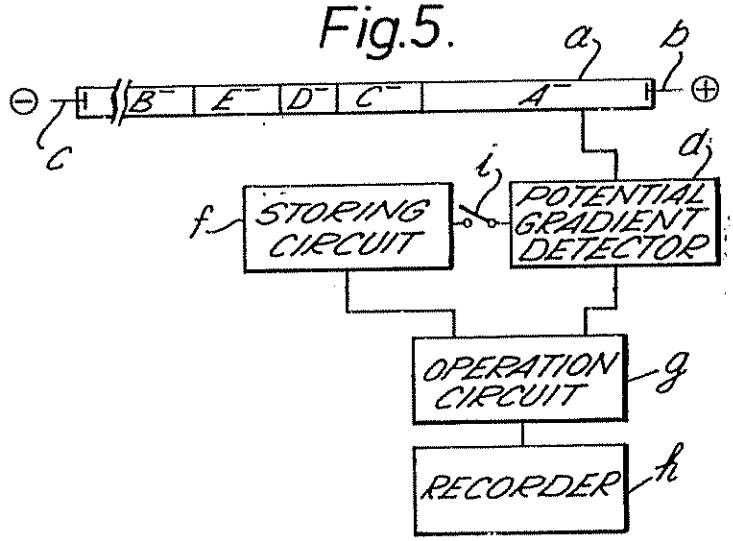
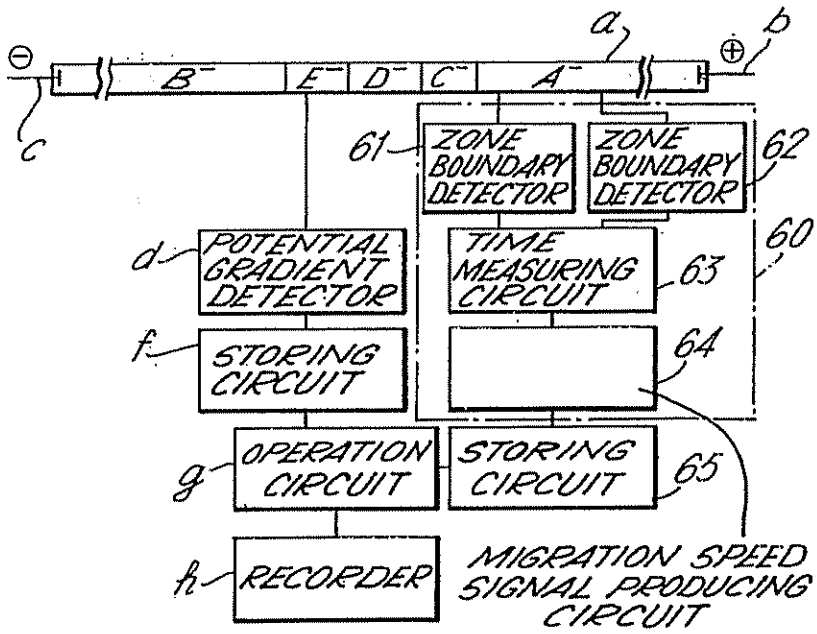

a
b
B⁻
E⁻
D⁻
C⁻
A⁻
c
d
i
f
STORING CIRCUIT
POTENTIAL GRADIENT DETECTOR
OPERATION CIRCUIT
g
RECORDER
h

Fig.6.


a
b
c
B⁻
E⁻
D⁻
C⁻
A⁻
61
ZONE BOUNDARY DETECTOR
ZONE BOUNDARY DETECTOR
62
60
d
POTENTIAL GRADIENT DETECTOR
TIME MEASURING CIRCUIT
63
f
STORING CIRCUIT
64
g
OPERATION CIRCUIT
STORING CIRCUIT
65
h
RECORDER
MIGRATION SPEED SIGNAL PRODUCING CIRCUIT

APPARATUS FOR ELECTROPHORETIC ANALYSIS

This invention relates to an apparatus for electrophoretic analysis of ions or like electrically charged particles.

Isotachophoretic analysis is generally conducted in the following manner. A leading electrolytie, a terminal electrolyte and a sample solution between the two electrolytes are introduced into a capillary tube to form a liquid column therein, to which an electric field is applied along the length of the liquid column for separation of the component ions of the sample. In this case, if the ions or other electrically charged particles in the sample to be analyzed (which will be referred to simply as the ions) are cations, for example, the leading electrolyte contains a single kind of cations having a higher mobility than that of the sample ions while the terminal electrolyte contains a single kind of cations having a lower mobility than that of the sample ions.

The cations in the sample solution are separated as they migrate toward the cathode and when they have been completely separated, different zones are formed lying in the order of their respective mobilities and then these zones migrate at a constant speed. The width (that is, the thickness in the direction of migration) of each zone is proportional to the number of the ions contained therein. Therefore, by measuring the width of each of the completely separated zones it is possible to quantitatively determine the sample ions. The width of the zone can be measured by detecting the boundary between adjacent zones containing different kinds of ions.

Various types of detectors have hitherto been used for detecting the zone boundaries, such as those which depend for detection of the zone boundaries upon difference in absorption of ultra-violet rays by different zones or difference in the amount of heat pproduced due to the difference in the amount of heat produced due to the difference in the resistance between different zones, and those which are so designed as to detect the potential gradient along the liquid column in the capillary tube. The latter type, which is generally referred to as the potential gradient detector, has come to be in wide use due to its high sensitivity and versatility.

As is well known, in isotachophoresis the migration current is always at a constant level and determines the migration speed of the ion, so that so long as the migration current is kept at a constant level, the migration speed of the ion is kept constant.

On the other hand, the migration speed S of the ion can be expressed as the product of the inherent mobility M of the ion and the potential gradient V thereof as follows:

$$S = M \cdot V \tag{1}$$

If the speed S is constant, the above equation is expressed as:

$$MV = k \tag{2}$$

This means that the mobility is in inverse proportion to the potential gradient V.

Suppose that different kinds of ions contained in a sample have mobilities M1, M2, M3 . . . Mn and potential gradient values V1, V2, V3, . . . Vn, respectively, the following equation is obtained provided that the migration speeds of the ions are equal.

$$M1V1 = M2V2 = M3V3 \ldots = MnVn = k \tag{3}$$

From this equation we obtain

$$\frac{V2}{V1} = \frac{M1}{M2}, \quad \frac{V3}{V1} = \frac{M1}{M3}, \quad \frac{V4}{V1} = \frac{M1}{M4}, \quad \frac{Vn}{V1} = \frac{M1}{Mn} \tag{4}$$

It is obvious from the equation (4) that the ratio of the potential gradient of one of the different kinds of sample ions to that of another kind is equal to the ratio of the mobilities of the two kinds of sample ions, and these values are independent of the migration speed. Therefore, if the potential gradients of the sample ions in the separated zones are measured so that with the potential gradient (e.g., V1 in equation (4)) of one particular kind of the sample ions being chose as a standard or reference, if the ratio of each of the potential gradients (V2, V3, . . . and Vn) of the other sample ions to the standard value V1 is calculated, it is possible to obtain values related to the mobilities of the sample ions and from these values the sample components being measured can be identified.

Accordingly, the primary object of the invention is to provide an apparatus for electrophoretic analysis of samples, which is capable of obtaining a value relating to the mobility specific to each of the separated sample ions so that the sample ions can easily be identified. To this end, in accordance with the invention the potential gradient of a standard or reference ion and those of the sample ions are measured and the ratio of the potential gradient of each of the sample ions to that of the reference ion is calculated.

In one embodiment of the invention the apparatus comprises a first detector for detecting the potential gradient of a leading ion, a second detector for detecting the potential gradients of the different kinds of ions contained in a sample, the first and second detectors being arranged alongside the capillary tube in which isotachophoretic separation of the sample ions is to be effected, an operation circuit for performing a predetermined operation on the outputs from the two detectors to produce an output corresponding to the ratio of the potential gradient of each of the different kinds of sample ions to that of the leading ion; and an indicator for indicating the output from the operation circuit.

In another embodiment of the invention the apparatus comprises a detector for detecting the potential gradient of each of different kinds of ions, a circuit for storing the output from the detector corresponding to the potential gradient of a particular kind of ions, an operation circuit for the outputs from both the storing circuit and the detector to produce an output corresponding to the ratio of the potential gradient of each of the different kinds of ions to that of said particular kind of ions, and an indicator for indicating the output from the operation circuit.

As previously mentioned, the migration speed S of a certain kind of ions is given by the product of the mo-

bility M and the potential gradient V thereof, that is, $S = M \cdot V$ Therefore, the mobility M can be expressed as

$$M = S/V \quad (5)$$

If the migration speed and the potential gradient of a sample ion are measured, it is possible to calculate the mobility specific to the ion and consequently identify the ion.

Another object of the invention is, therefore, to provide an apparatus for electrophoretic analysis of ions or like charged particles, which is capable of directly obtaining the mobility of a sample ion immediately after it has been separated, so that the sample ion can be identified. To this end the apparatus of the invention comprises a detector for detecting the potential gradient of each separated ion to produce a corresponding output signal, a detector for detecting the migration speed of the ion to produce a corresponding output signal, an operation circuit for conducting a predetermined operation on the outputs from the detectors to produce a corresponding output signal, and an indicator for indicating the output from the operation circuit.

The invention will be explained in detail with reference to the accompanying drawings, wherein:

FIG. 1 is a schematic view of an apparatus for electrophoretic analysis;

FIG. 2 is a sectional view of a potential gradient detector used in the apparatus of FIG. 1;

FIG. 3A is a schematic representation of separated ions in a capillary tube;

FIG. 3B shows the potential distribution along the capillary tube;

FIG. 3C shows the potential gradients and mobilities of the separated ions;

FIG. 3D shows the differentiated values of the potential gradients; and

FIGS. 4 to 6 are schematic views of different embodiments of the apparatus of the invention.

Referring in detail to the drawings, first to FIG. 1, there is shown a capillary tube 1, which is made of an insulating material such as Teflon. The inner and outer diameters of the tube are, for example, 0.5 mm and 2 mm, respectively. A chamber or cell 2 enclosing a terminal electrode 21 is connected to one end of the capillary tube, to the other end of which another chamber or cell 3 enclosing a leading electrode 31 is connected. The two electrodes 21 and 31 are connected through lines 41 and 42 to the negative and positive output terminals of a source of electricity 4, which may comprise a current stabilized power supply which provides an adjustable constant output direct current, with a neutral tap being preferably grounded.

A pair of conduits 51 and 52 connect the terminal and leading electrode chambers 2 and 3 to a pair of tanks 54 and 53, respectively, adjacent the bottom portions thereof, which contain terminal and leading electrolytes, respectively. Stop valves 57 and 58 are inserted in the conduits 51 and 52 to control the supply of the electrolytes from the tanks 53 and 54 to the chambers 2 and 3. A pipe 55 connects the upper portions of the tanks 53 and 54 to a sourse 56 of presurized inert gas such as helium.

The capillary tube is provided with a sample introducing device 22 through which a leading electrolyte are introduced into the capillary tube to form a liquid column therein in the well known manner.

A heating chamber 7 encloses the capillary tube 1 to keep the liquid column at a predetermined temperature, which is controlled by a source of electricity 71.

Adjacent one end of the capillary tube there is provided a detector 8, one example of which is shown in FIG. 2 as a potential gradient detector. The detector comprises a holding block 81 made of a transparent insulating material such as methacrylic resin, and along the axis of the block 81 there is provided a hollow cylindrical detecting chamber 82 having its opposite ends connected to the end portions 11 and 12 of the capillary tube 1.

The end portions of the tube are secured to and supported by the block 81 by means of screw plugs 84 with a seal tape 83 sealing between the adjacent members. A pair of electrodes 85 and 86 for detecting the potential gradient, made of a platinum wire having a diameter of about 0.08 mm, extend normal to the axis of the detecting chamber through a pair of holes formed in the block 81 as far as the inner ends of the electrode wieres are exppосed at, but not projecting from, the inner surface of the detecting chamber 82, with the other ends of the electrodes being soldered to the bare ends of cable wires 87 held by the block 81. The space about the electrodes and the bare ends of the cable is filled with a suitable insulating adhesive material 88 such as Araldite (a trademark of CIBA).

It should be noted as the important features of the detector shown in FIG. 2 that, firstly, the inner diameter of the detecting chamber 82, e.g. 0.8 mm, is somewhat greater than that of the capillary tubes 11 and 12, e.g. 0.5 mm and, secondly, the electrodes 85 and 86 comprise as thin wires as possible the inner ends of which do not project into the detecing chamber 82 but agree with the inner wall surface thereof.

Due to the first feature the current density becomes lower in the detecting chamber than in the capillary tubes 11, 12 and due to the second feature the area of the electrodes which is in contact with the liquid in the detecting chamber is minimized so that discharge of the ions at the electrode surface and consequent formation of bubbles and attachement of deposits to the electrodes can be suppressed to a great extent.

Returning to FIG. 1, the sensing electrodes 85 and 86 are connected through a pair of cables 87 to an impedance converter 91 having a high input impedance and a low output impedance. The output of the impedance converter 91 is applied to a voltage-to-frequency converter 92 which produces a pluse signal the frequency of which is proportional to the output of the impedance converter 91. A first energy converter 93 such as a light emitting diode receives the output electrical signal from the converter 92 to convert it to a corresponding optical or other electromagnetic wave signal. A channel 94 transmits the electromagnetic wave from the first energy converter 93 to a second energy converter 95 such as a phototransistor or phototube which converts the optical signal again to a corresponding electrical pulse signal. The electrical signal is applied to a frequency-to-voltage converter 96 which produces a voltage corresponding to the frequency of the electrical signal received from the converter 95.

The output from the frequency-to-voltage converter 96 is applied to a signal processing circuit 97 including a differentiator and other elements not shown, the output from which is applied to a recorder 98, which may be replaced by an indicator, a displayer or any other suitable device for measuring the zone width.

An insulating means **99** isolates the circuits **91, 92** and **93** from the ground so as to prevent any leakage current from flowing between the sensing electrodes and the ground. The arrangement helps completely isolete the sensing portion from the fixed potential source such as the ground so that no leakage current flows between the sensing electrodes and the exterior parts thereby preventing formation of bubbles.

In operation, a leading electrolyte, a sample solution and a terminal electrolyte are introduced into the capillary tube, with the sample being interposed between the two electrolytes. When the output from the source **4** is impressed across the electrodes **21** and **31**, a potential gradient is produced along the liquid column formed in the capillary tube **1**, so that the ions, say, anions in the sample migrate toward the positive electrode **31**.

The different kinds of anions in the sample are gradually separated into different zones according to their inherent mobilities as they move at their respective migration speeds which are betwwen the migration speed of the anions of the leading electrolyte and that of the anions of the terminal electrolyte. When the separation has been completed, each of the separated zones includes a different single kind of ions contained in the sample and the widths of the zones are proportional to the amounts of the sample ions introduced. After that the zones move at the same speed.

FIG. **3A** schematically shows the zones completely separated in the above manner. In FIG. **3A** the symbols + and − designates the polarities of the voltage impressed across the liquid column in the capillary tube; the numeral **8** designates the potential gradient detector; and the symbols A^- and B^- designate the leading and the terminal ions, respectively, with different kinds of sample ions C^-, D^- and E^- in the separated zones between the leading and the terminal electrolytes.

FIG. **3B** shows the potential distribution of each ion in the capillary tube at a time corresponding to the separated condition shown in FIG. **3A**, and FIG. **3C** shows the potential gradient values VA, VB, VC and VE and mobilities MA, MC, MD and ME of the ions. This means that the potential gradient values detected by the detectors **8** as the separated zones pass the detector one after another will draw the same curve as shown in FIG. **3C** provided that the capillary tube has a sufficient length between the sample introducing device **22** and the detector **8** for the sample ions to be completely separated.

If the output from the detector **8** is differentiated so that the differentiated signal is plotted aginst time, a graph as shown in FIG. **3D** is obtained. By the distance between adjacent differentiated signal peaks it is possible to know the width of each zone and consequently determine the quantity of each component of the sample, and by the difference in height between adjacent peaks it is possible to known the presence of a difference in mobility between the sample ions in adjacent two separated zones. However, these informations can be obtained only after the data on the recorder chart have been analyzed, and such analysis requires somewhat troublesome work.

According to this invention, the ratio of the potential gradient of each sample ion to that of a specific or reference ion is calculated, and directly from the ratio the sample ion can be identified.

FIGS. **4** and **5** schematically show different embodiments of the invention. First in FIG. **4** a pair of electrodes *b* and *c* apply a constant current to an electrophoretic capillary tube *a*, within which leading ions A^- and terminal ions B^- and three different kinds of sample ions C^-, D^- and E^- now completely separated migrate at a constant speed toward the anode *b*.

A pair of first and second potential gradient detectors *d* and *e* are provided on the capillary tube, the detector *d* being at the side of the anode and the detector *e* being at the side of the cathode. The two detectors detect the potential gradients of the ions A^-, C^-, D^- and E^- to produce corresponding output signals. A storing circuit *f* is connected to the output of the detector *d* to store the detected potential gradient of the leading ion A^-. An operation circuit *g* is connected to the output of the storing circuit *f* and that of the second detector *e* to calculate the ratios VC/VA, VD/VA and VE/VA, wherein Va is the potential gradient of the leading ion A^- and VC, VD and VE are those of the sample ions C^-, D^- and E^-.

As previously mentioned, these ratios are equal to the ratios of the mobility MA of the leading ion A^- to those MC, MD and ME of the sample ions C^-, D^- and E^-, that is, MA/MC, MA/MD and MA/ME, respectively. Since the ratios VC/VA, VD/VA and VE/VA are constant regardless of the measuring conditions such as the migration current, the diameter of the capillary tube, the concentrations of the electrolytes, etc., each of the ratios VC/VA, VD/VA and VE/VA expresses a value specific to the corresponding component ion of the sample.

Therefore, by indicating these values on a recorder or indicator h it is possible to obtain values relating to the mobilities specific to the sample components.

Turning to FIG. **5** which shows another embodiment of the invention, and wherein the same reference numerals denote corresponding parts, a potential gradient detector *d* detects the potential gradients of the leading ion A^- and the sample ions C^-, D^- and E^- succesively, and a storing circuit *f* is connected through a switch *i* to the detector *d* so as to store the potential gradient VA of the leading ion A^-. An operation circuit *g* is connected to the output of the storing circuit *f* and that of the detector *d* so as to calculate the ratios VC/VA, VD/VA and VE/VA of the potential gradients VC, VD and VE of the sample ions successively detected by the detector *d* to the potential gradient VA of the leading ion.

In the above description, the ratio of the potential gradient of each sample ion to that of the leading ion is calculated, but the arrangement may be such that one of the different kinds of sample ions is chosen as the standard or reference ion and the ratio of the potential gradient of each of the other kinds of ions to that of the reference ion is calculated.

The following table shows the results of an experiment conducted in accordance with the invention. Chloride ion is used as the leading ion and chosen as the standard or reference and the ratios of the potential gradients of the other ions to that of the reference ion are obtained, while the inverse ratios of the mobilities of these ion are obtained theoretically from the mobilities of the ions. The migration current is 100 μA and the pH is 6.0.

	Ratios of potential gradients	Inverse ratios of mobilities (theoretical values
Chloride ion	1 00	1 00

-continued

Sulphate ion	1.20	1.30
Nitrate ion	1.35	1.39
Oxalate ion	1.50	1.45
Formate ion	1.90	1.82
Citrate ion	2.10	1.99
Maleinate ion	2.50	2.42
Acetate ion	4.10	—
Gultamate ion	8.00	—

As shown in the table, the ratios of the potential gradients are slightly different from the inverse ratios of the mobilities although theoretically these two ratios must be equal.

The cause for the discrepancy may be due to errors in the experiments which have been caused by the low precision of the measuring device or by the fact that the mobility of each ion, unlike its atomic or molecular weight, considerably varies with the pH of the solution, the ambient temperature and other measurement conditions. However, the discrepany is negligible in practice in identification of various ions and can be reduced by improving the precision of the measuring device.

FIG. 6 schematically shows a third embodiment of the invention for directly obtaining the mobility of each separated sample ion from the potential gradient and the migration speed thereof. In FIG. 6 the same reference symbols as in FIGS. 4 and 5 denote corresponding parts so that explanation of these parts will be omitted.

In addition to the potential gradient detector *d*, the capillary tube *a* is provided with a migration speed detector generally designated by **60**. The detector **60** comprises: a pair of zone bundary detectors **61** and **62** arranged a predetermined distance apart from each other along the length of the capillary tube; a time measuring circuit **63** controlled by the output signals from the detectors **61** and **62**; and a migration speed signal producing circuit **64** which receives the output from the time measuring circuit **63** to produce an output signal corresponding to the migration speed of the ion that has passed the zone boundary detectors **61** and **62**. The migration speed signal from the circuit **64** is stored in a storing circuit **65**.

The output from the potential gradient detector *d* is stored in a storing circuit *f*, the output from which and the migration speed signal from the storing circuit **65** are applied to an operation circuit *g* which conducts the calculation of S/V to produce an output signal corresponding to the mobility M of the ion. The signal expressing the mobility of the ion is indicated by recorder or indicator *h*.

In operation, the potential gradient detector *d* detects the potential gradient of one of the separated ions after another as they move through the capillary tube toward the anode, and the detected potential gradient values are successively stored in the storing circuit *f*.

On the other hand, if the zone boundary detectors **61** and **62** are so designed as to produce an output in response to a change in the potential gradient, when the boundary between the zone containing the A^- ions and the zone containing the C^- ions passes the detector **61**, this detector produces an output pulse whereupon the time measuring circuit **63** is started to produce an output signal until the same boundary passes the detector **62**, whereupon the detector **62** produces an output pulse to terminate the output from the time measuring circuit **63**. In response to the output from time measuring circuit **63**, the circuit **64** calculates the migration speed of the ions C^- to produce a corresponding signal.

Since the different kinds of ions have the same migration speed, the migration speed signal is stored in the storing circuit **65**, so that from this migration speed and each of the potential gradient values of the different kinds of ions the mobility specific to each of the different kinds of ions is calculated and then indicated on the indicator *h*.

As mentioned just above, since the different kinds of ions have the same migration speed, if the time required for the boundary between the zones of the A^- and C^- ions to move from one of the two zone boundary detectors to the other is measured and memorized, it is not necessary to detect the migration speed again.

Various modifications and changes of the arrangements of the invention are possible. For example, in FIG. 6 the storing circuit *f* may be omitted; and one of the two zone boundary detectors may be replaced by the potential gradient detector.

What I claim is:

1. Apparatus for electrophoretic analysis of ions and like electrically charged particles comprising: a capillary tube; means for providing within said tube a liquid column containing different kinds of ions; means for providing a potential difference between the opposite ends of said liquid column to separate said ions into different zones; means for detecting the potential gradients of said ions in said separated zones; and operation circuit means for calculating the ratio between the potential gradient of one of said different kinds of ions and that of each of the other kinds of ions.

2. The apparatus of claim **1**, further including means for indicating the output from said operation circuit means.

3. The apparatus of claim **2**, wherein said liquid column comprises a leading electrolyte containing leading ions, a terminal electrolyte containing terminal ions and a sample solution containing a single or different kinds of ions and interposed between said leading and terminal electrolytes.

4. The apparatus of claim **3**, wherein said detecting means comprises a first detector for detecting the potential gradient of said leading ions and a second detector for detecting the potential gradient of each of said sample ions; and wherein said operation circuit means calculates the ratio of the potential gradient of each of said different kinds of sample ions to that of said leading ions.

5. The apparatus of claim **1**, further including means for storing the potential gradient of a particular one of said different kinds of ions detected by said detecting means, and wherein said operation circuit means calculates the ratio between the potential gradient of said particular one kind of ions to the potential gradient of each of the other kinds of ions.

6. Apparatus for electrophoretic analysis of ions and like electrically charged particles, comprising; a capillary tube; means for providing within said capillary tube a liquid column containing different kinds of ions; means for producing a potential difference between the opposite ends of said liquid column to separate said ions into different zones; means for detecting the potential gradients of said different kinds of ions; means for detecting the migration speed of at least one of said different kinds of ions; operation circuit means for performing a predetermined operation on the outputs from said potential gradient detecting means and said

migration speed detecting means to produce an output corresponding to the mobility of each of said different kinds of ions; and means for indicating the output from said operation circuit means.

7. The apparatus of claim 6, wherein said liquid column comprises a leading electrolyte containing leading ions, a terminal electrolyte containing terminal ions and a sample solution containing a single or different kinds of ions and interposed between said leading and terminal electrolytes.

* * * * *

EXHIBIT 5

United States Patent [19]

Weinberger et al.

[11] **Patent Number: 5,066,382**

[45] **Date of Patent: Nov. 19, 1991**

[54] **THERMAL CONTROL FOR CAPILLARY ELECTROPHORESIS APPARATUS**

[75] Inventors: Scot R. Weinberger; James L. Mills, both of Reno, Nev

[73] Assignee: Spectra-Physics, Inc., San Jose, Calif.

[21] Appl. No.: 470,280

[22] Filed: Jan. 25, 1990

[51] Int. Cl.[5] G01N 27/26; B01D 57/02

[52] U.S. Cl. ..... 204/299 R; 204/180.1

[58] Field of Search ..... 204/299 R, 180.1, 183.3

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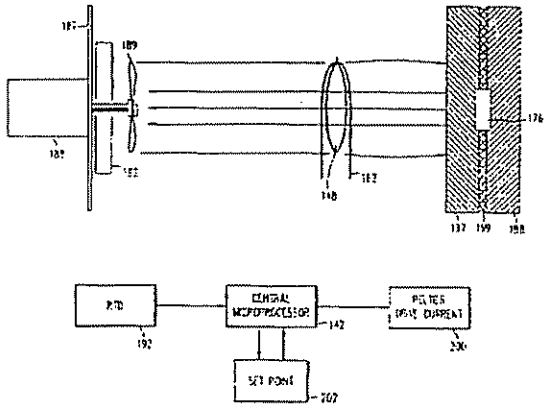
Primary Examiner—John F. Niebling
Assistant Examiner—John S. Starsiak, Jr.
Attorney, Agent, or Firm—Skjerven, Morrill, MacPherson, Franklin & Friel

[57] **ABSTRACT**

An electrophoresis instrument includes a capillary tube mounted in an air cooled cartridge. The cartridge also supports a spherical lens which is part of the optical detection apparatus. The cartridge rests in a manifold which includes the sample and buffer reservoirs. The temperature of the capillary tube is controlled by measuring the electrical resistance of the capillary tube during the electrophoresis process and then cooling or heating the cartridge by circulating temperature controlled air over the tube. The optical path associated with the instrument is a fiber optic bundle bifurcated close to dual detectors into a reference arm and a sample arm so as to provide similar reference and sample optical paths. The instrument may be used for temperature control for gradient electrophoresis and also, a neutral marker for determining electro-osmotic flow may be detected.

4 Claims, 15 Drawing Sheets



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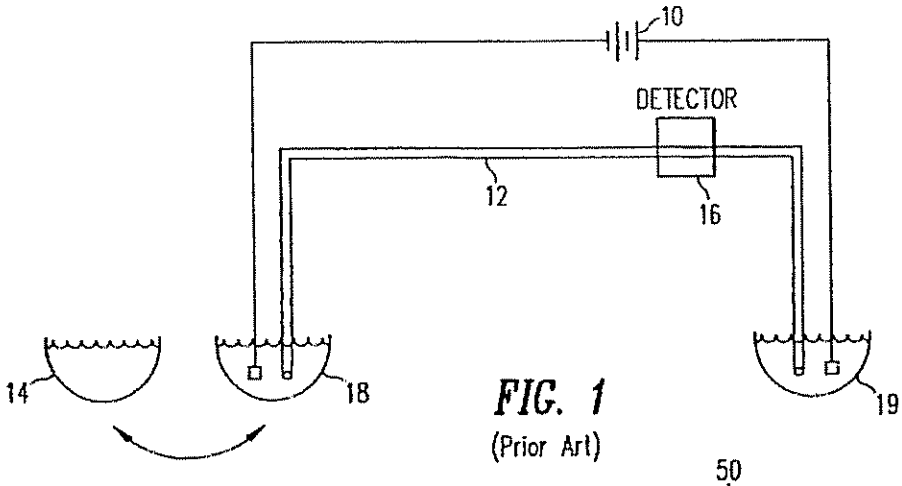

FIG. 1
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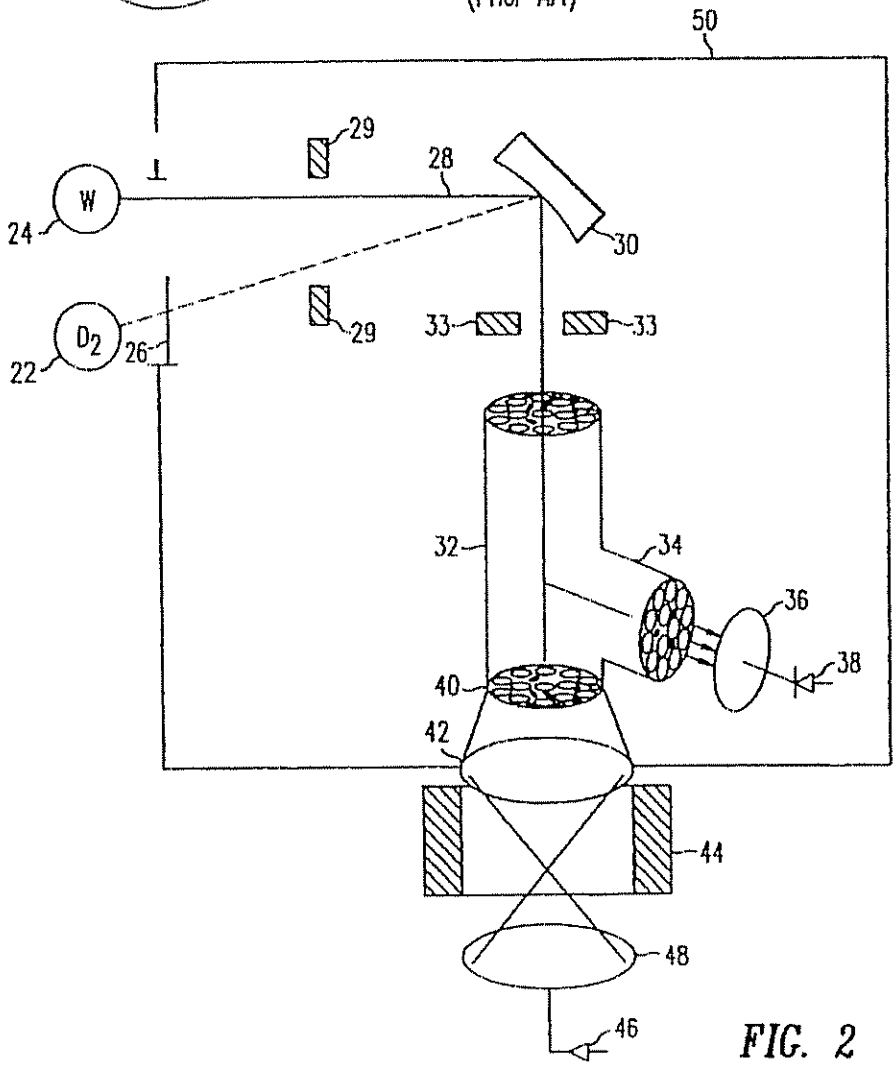

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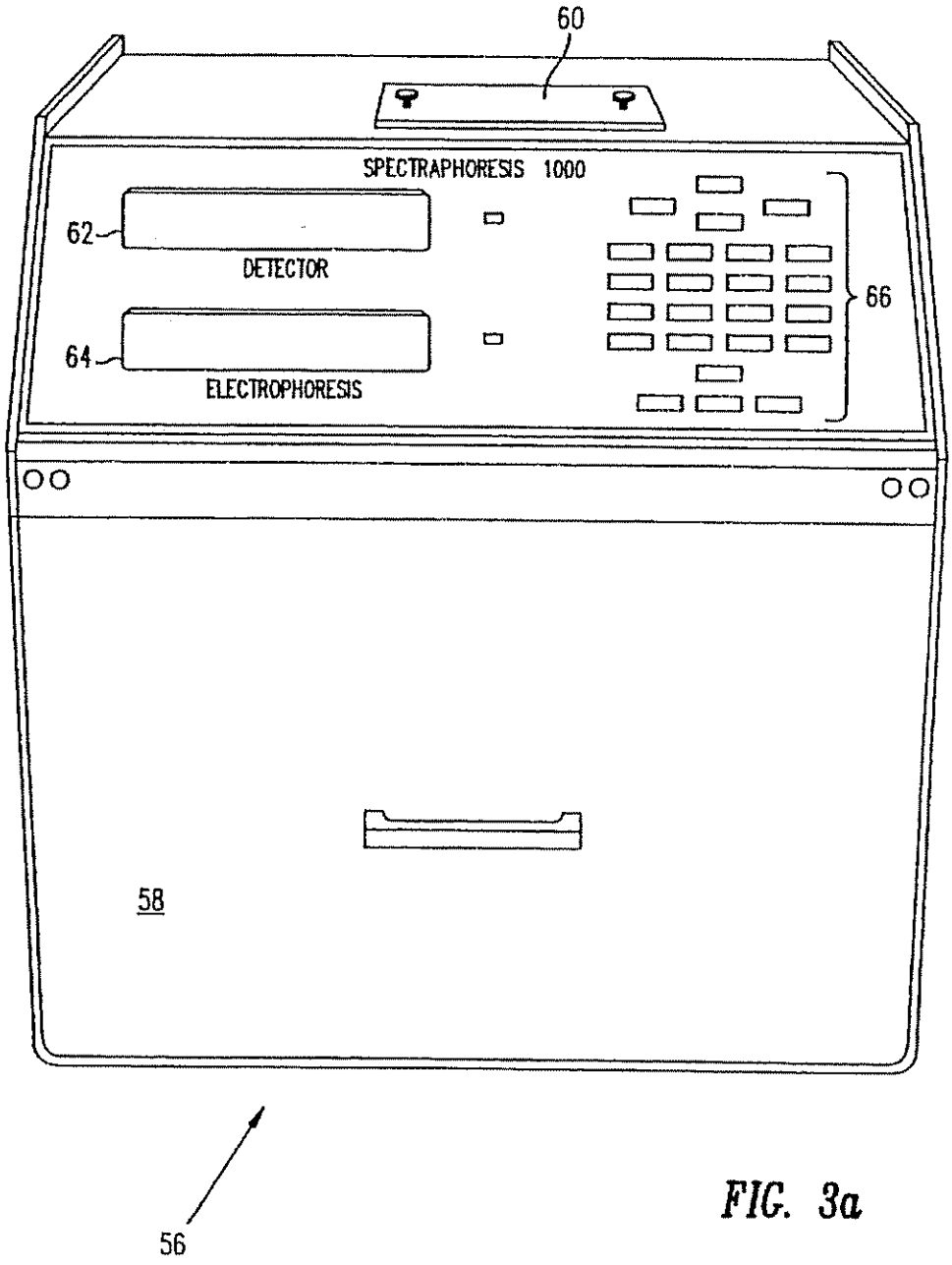
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FIG. 3a

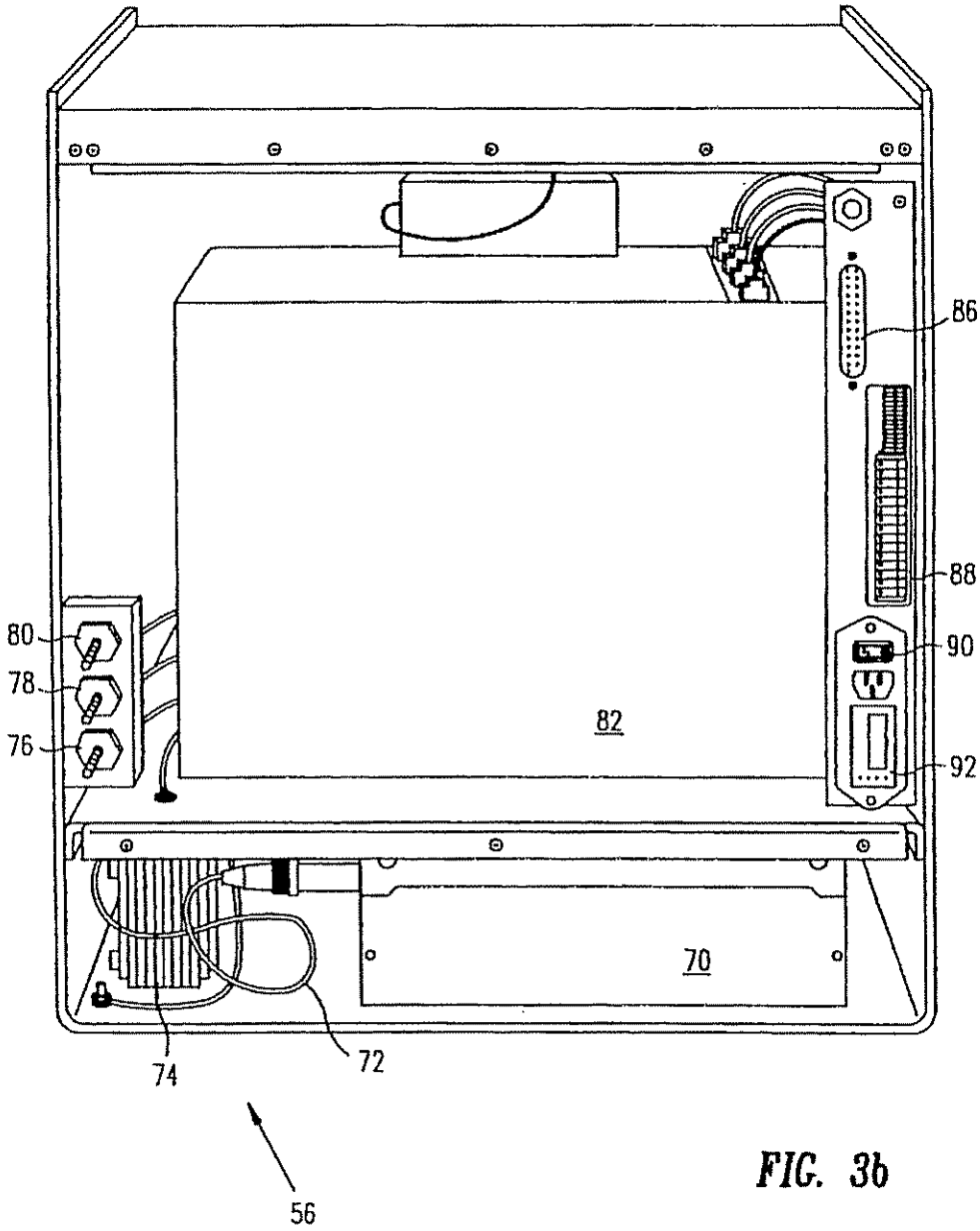

FIG. 3b

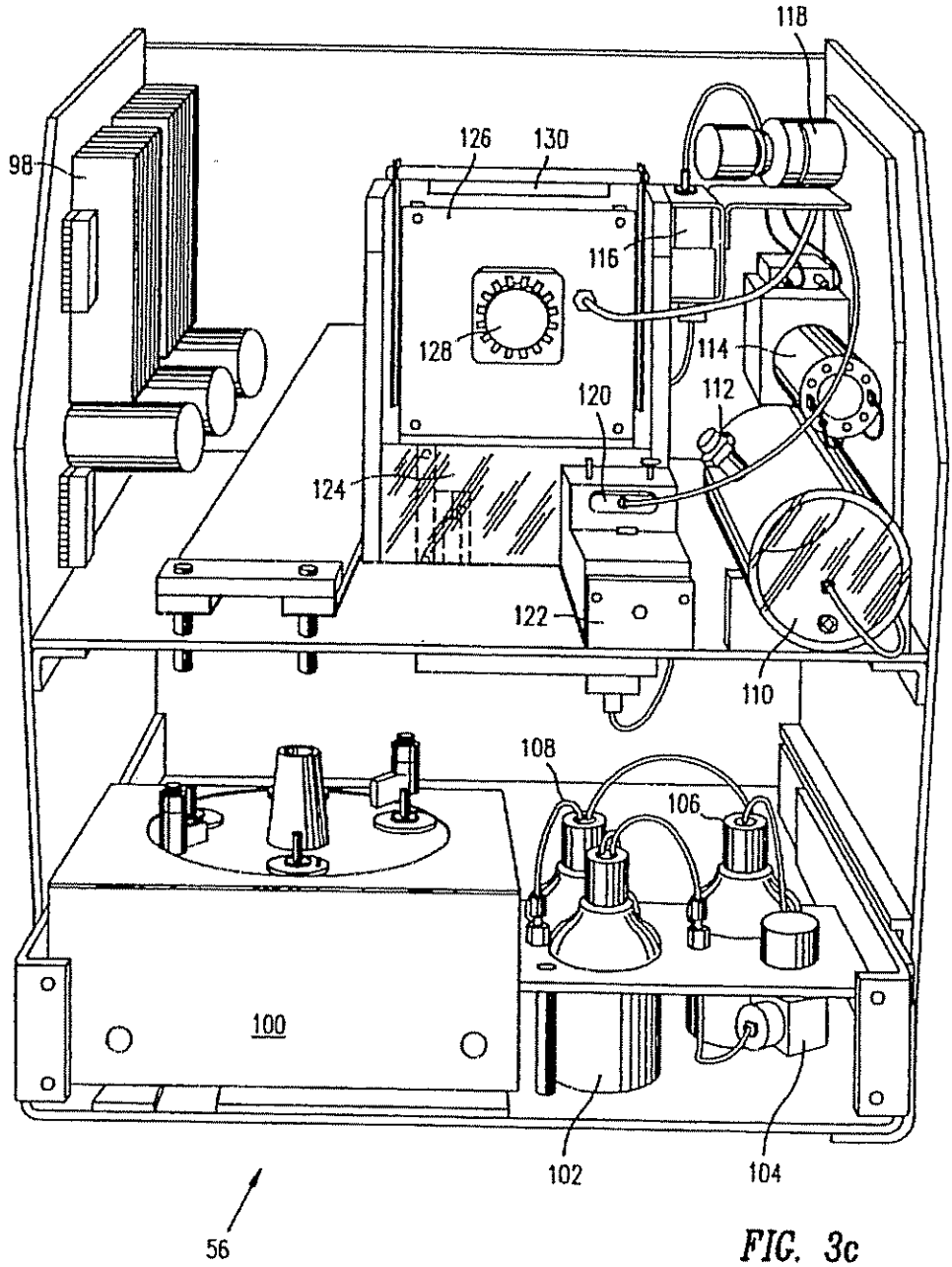
118
126
130
98
116
128
114
120
112
124
122
110
108
106
100
102
104
56

FIG. 3c

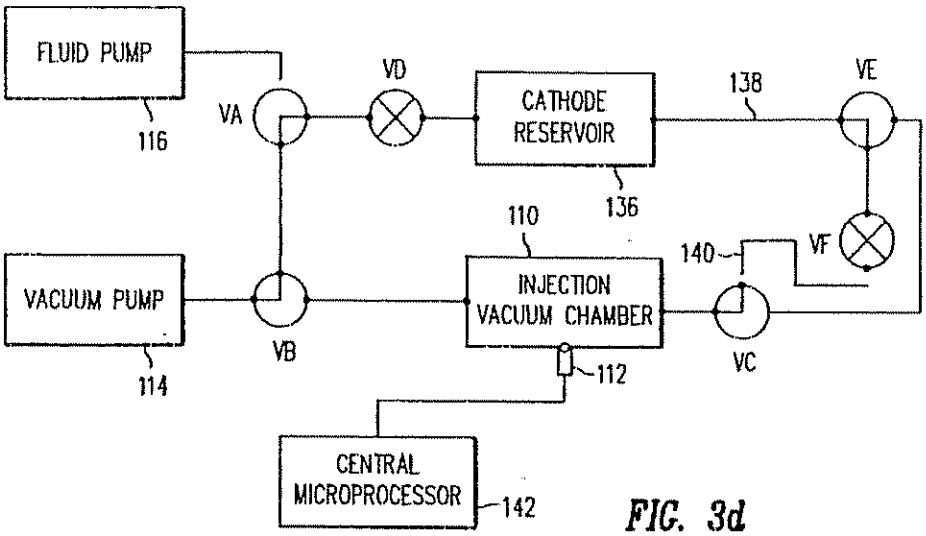
FLUID PUMP
116
VA
VD
CATHODE RESERVOIR
136
138
VE
110
VF
140
VACUUM PUMP
114
VB
INJECTION VACUUM CHAMBER
112
VC
CENTRAL MICROPROCESSOR
142

FIG. 3d

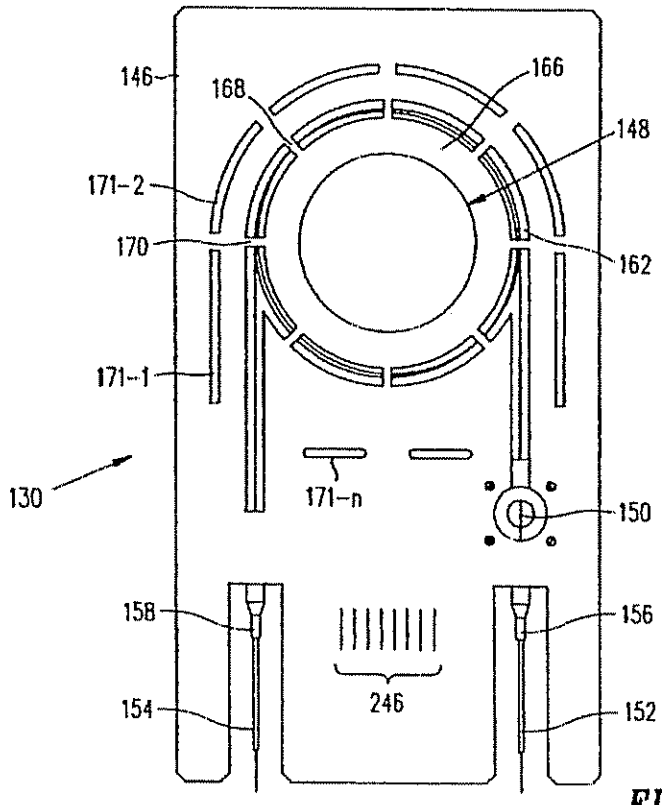
146
168
166
148
171-2
170
162
171-1
130
171-n
150
158
156
246
154
152

FIG. 4

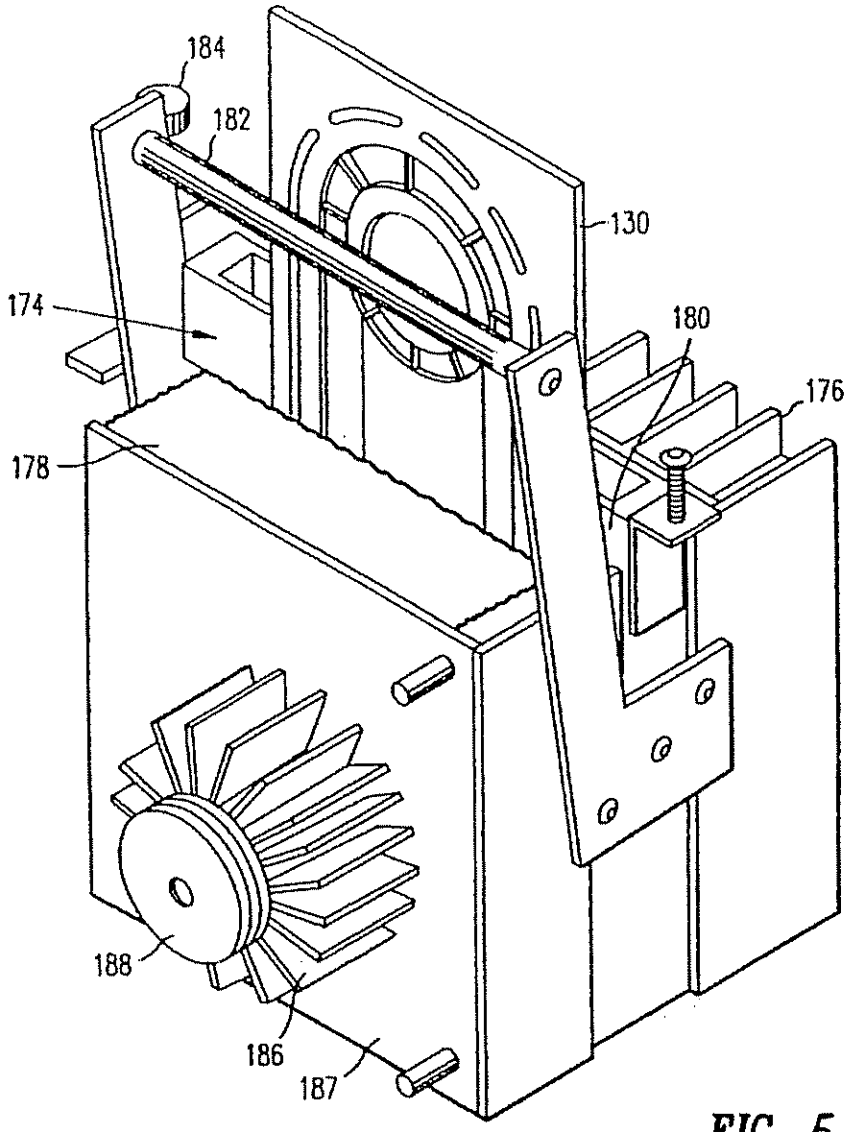

FIG. 5

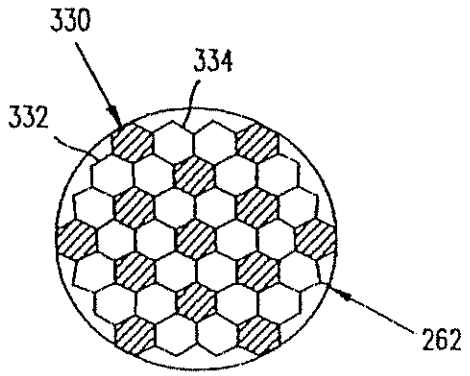

FIG. 10

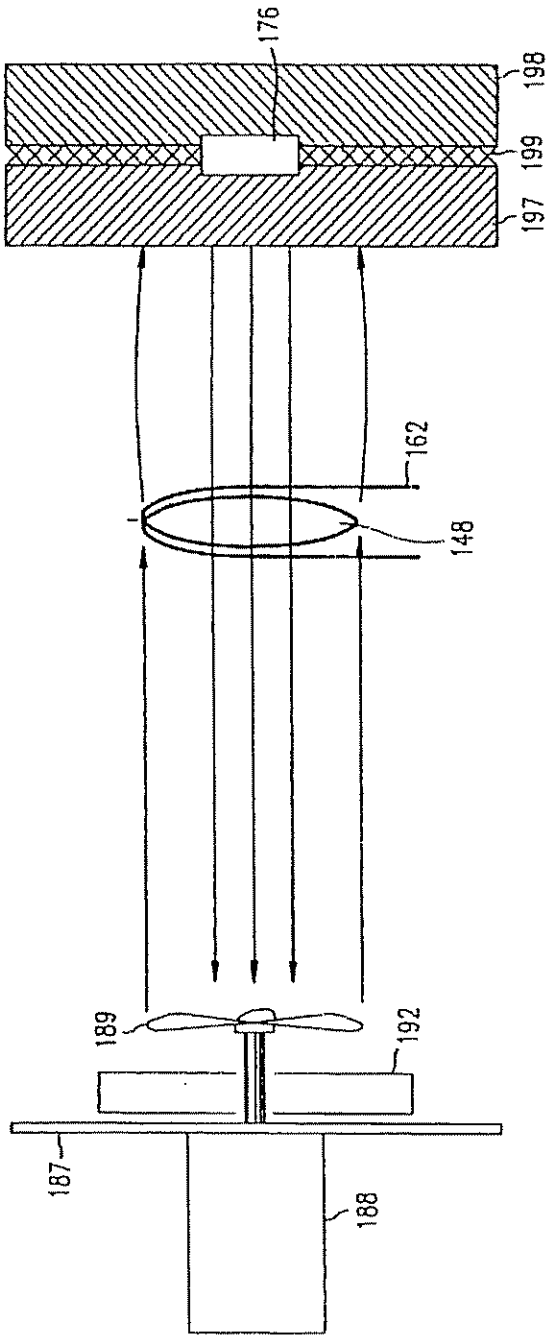
176
198
199
197
162
148
189
192
187
188

FIG. 6

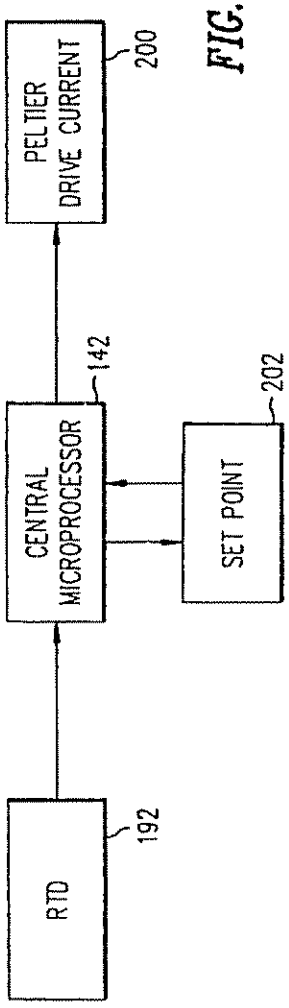
PELTIER DRIVE CURRENT
200
CENTRAL MICROPROCESSOR
142
SET POINT
202
RTD
192

FIG. 7

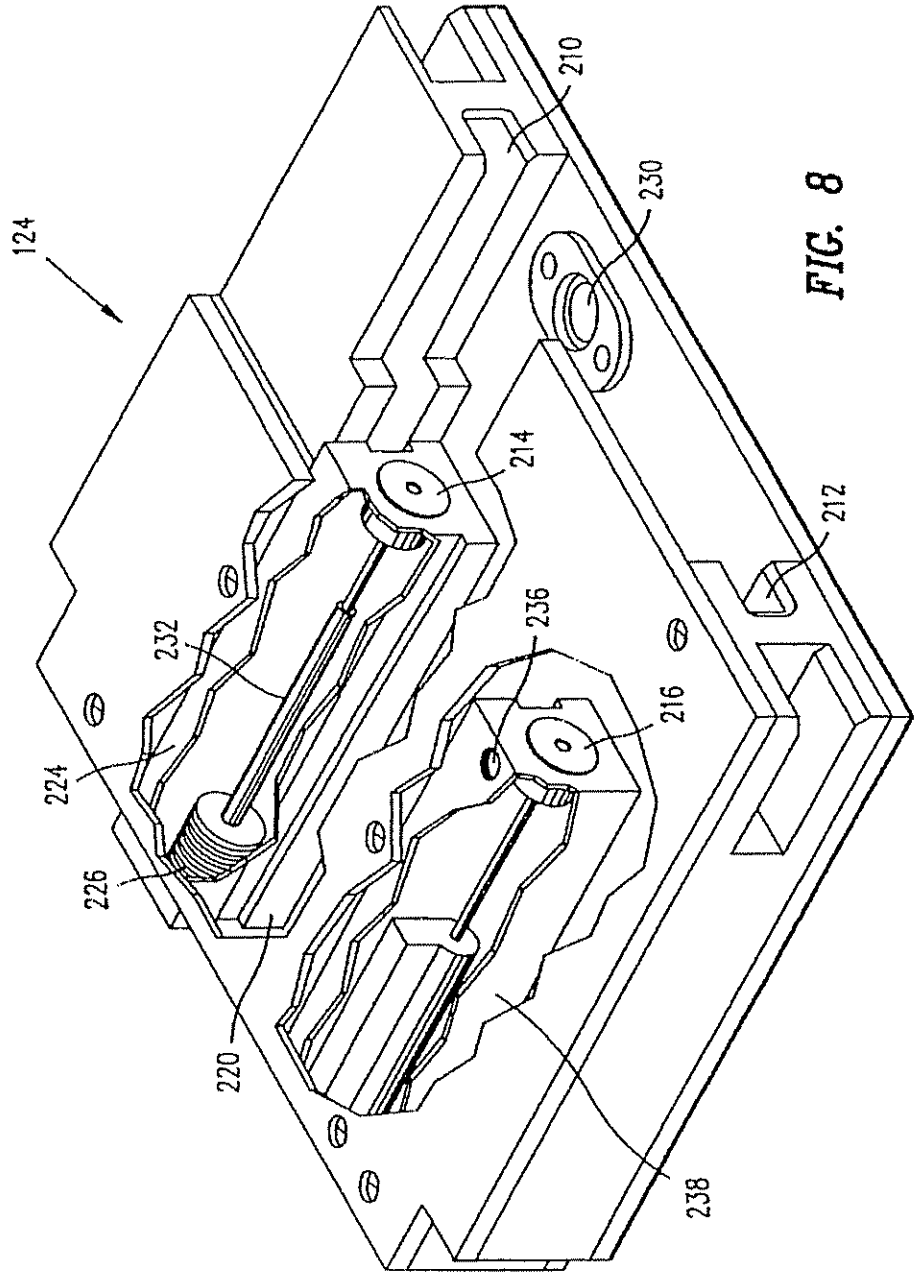

FIG. 8

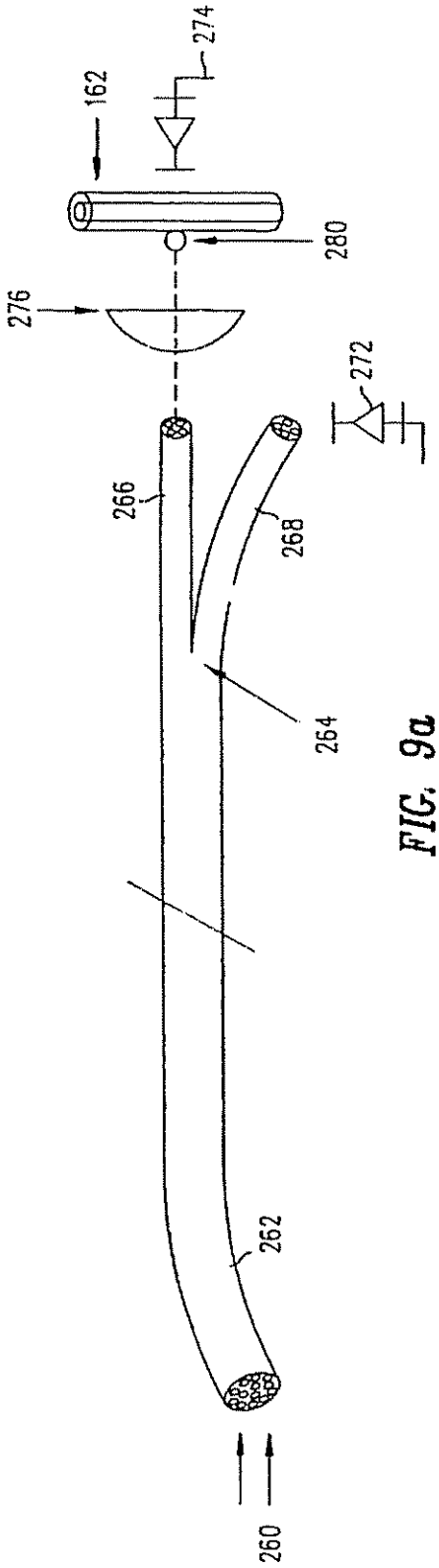
274
162
280
276
272
266
268
264
262
260

FIG. 9a

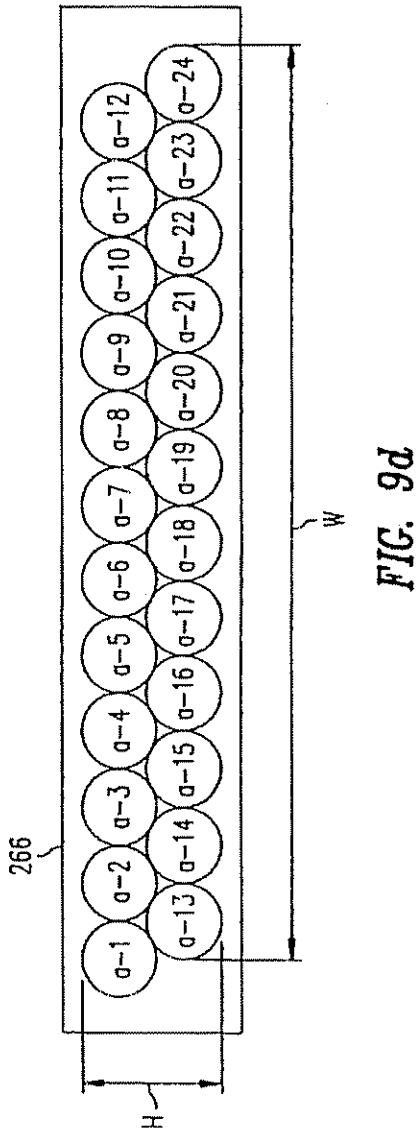
a-1
a-2
a-3
a-4
a-5
a-6
a-7
a-8
a-9
a-10
a-11
a-12
a-13
a-14
a-15
a-16
a-17
a-18
a-19
a-20
a-21
a-22
a-23
a-24
266
W
H

FIG. 9d

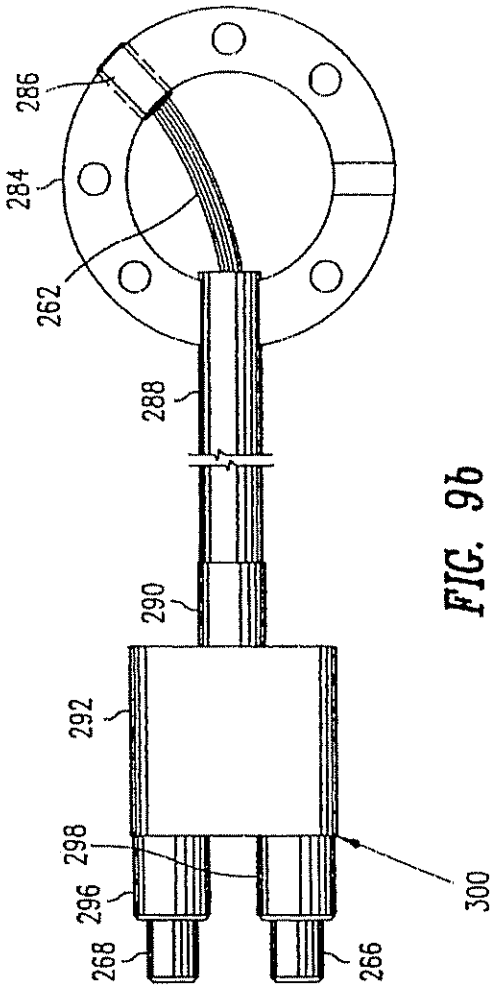

FIG. 9b

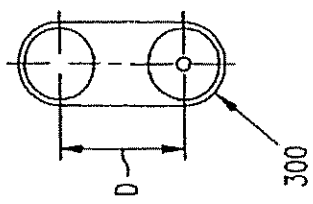

FIG. 9c

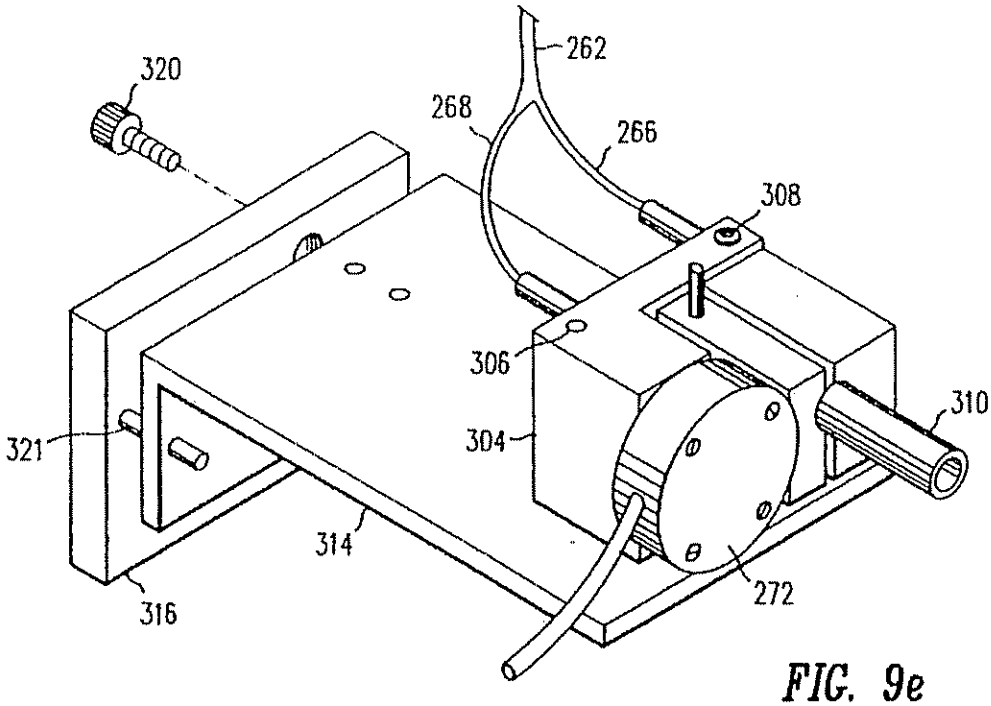

FIG. 9e

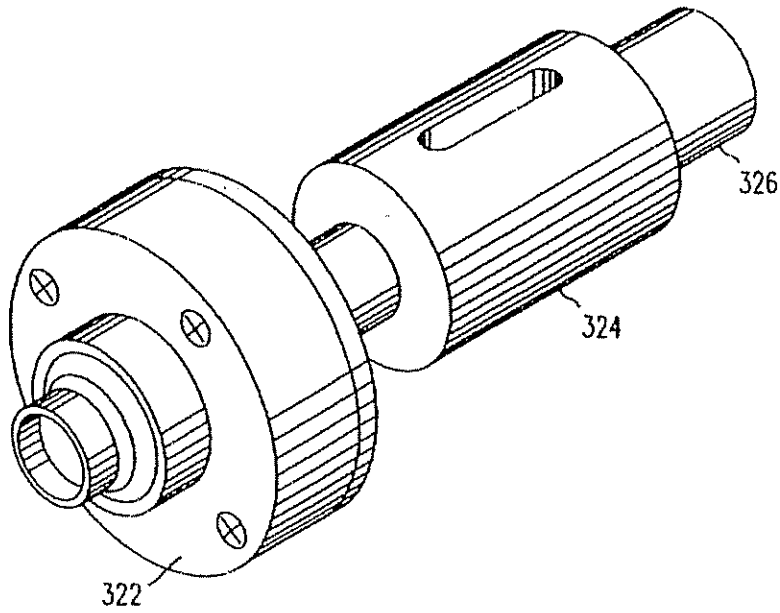

FIG. 9f

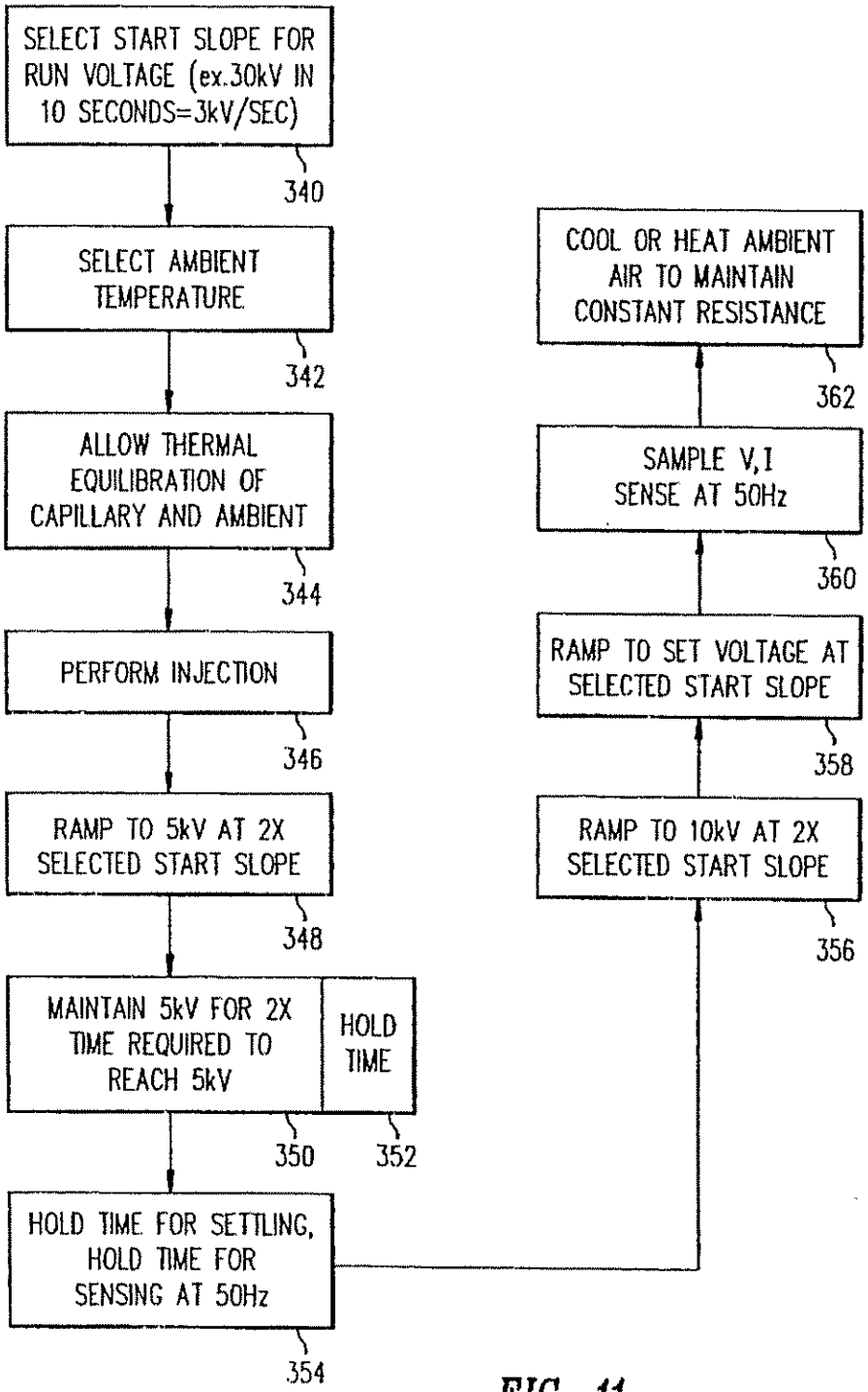
SELECT START SLOPE FOR RUN VOLTAGE (ex.30kV IN 10 SECONDS=3kV/SEC)
340
SELECT AMBIENT TEMPERATURE
342
ALLOW THERMAL EQUILIBRATION OF CAPILLARY AND AMBIENT
344
PERFORM INJECTION
346
RAMP TO 5kV AT 2X SELECTED START SLOPE
348
MAINTAIN 5kV FOR 2X TIME REQUIRED TO REACH 5kV
HOLD TIME
350
352
HOLD TIME FOR SETTLING, HOLD TIME FOR SENSING AT 50Hz
354
RAMP TO 10kV AT 2X SELECTED START SLOPE
356
RAMP TO SET VOLTAGE AT SELECTED START SLOPE
358
SAMPLE V,I SENSE AT 50Hz
360
COOL OR HEAT AMBIENT AIR TO MAINTAIN CONSTANT RESISTANCE
362

FIG. 11

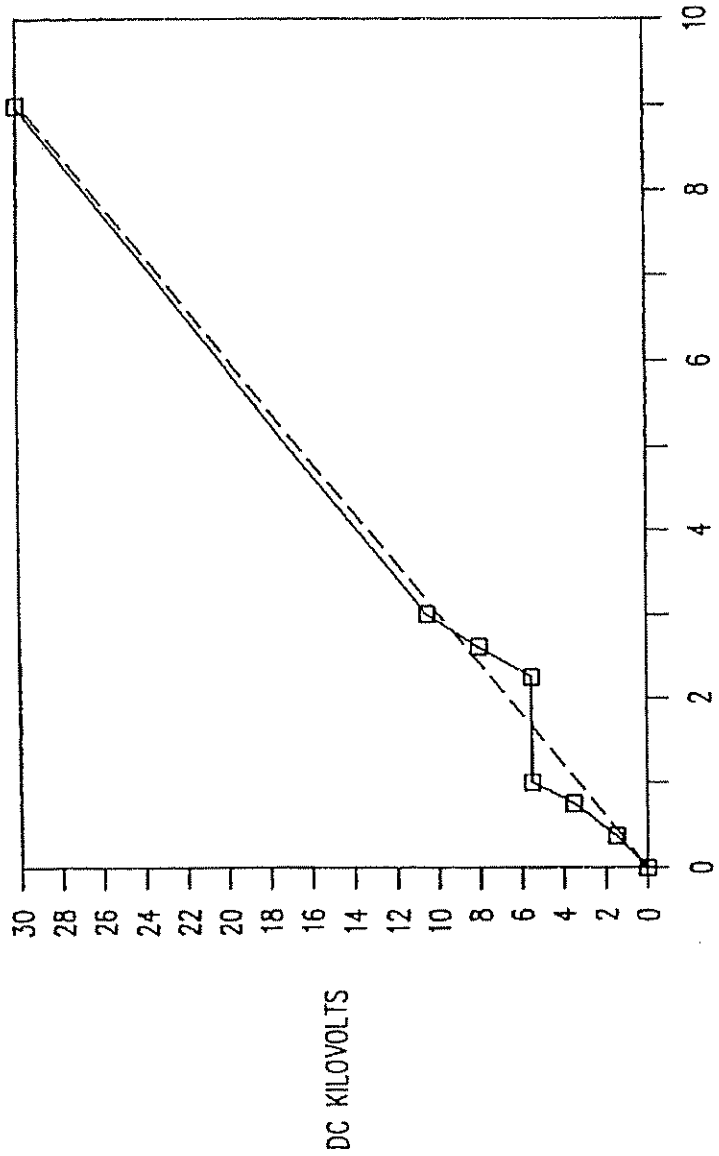
DC KILOVOLTS
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
TIME (SEC)
0
2
4
6
8
10

FIG. 12

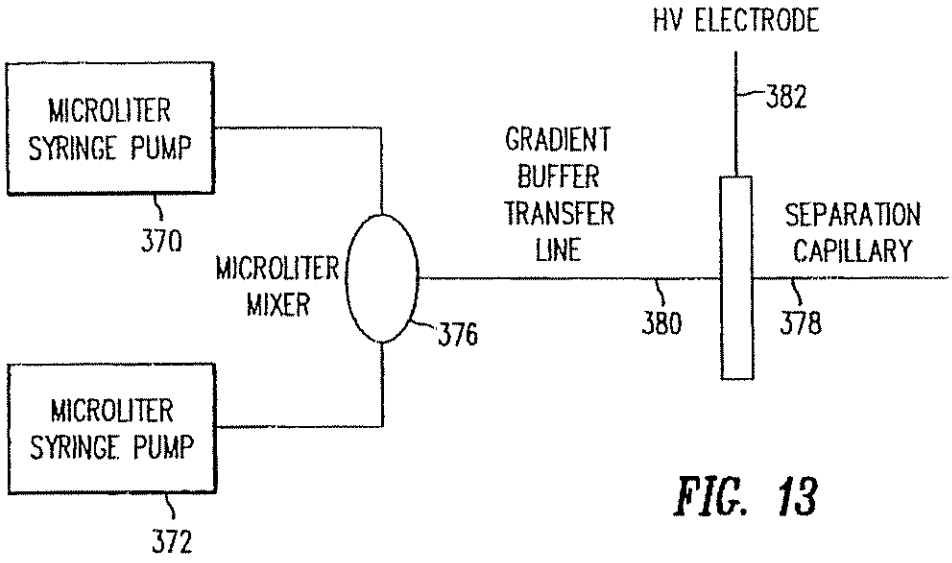
HV ELECTRODE
382
MICROLITER SYRINGE PUMP
370
MICROLITER MIXER
376
GRADIENT BUFFER TRANSFER LINE
380
SEPARATION CAPILLARY
378
MICROLITER SYRINGE PUMP
372

FIG. 13

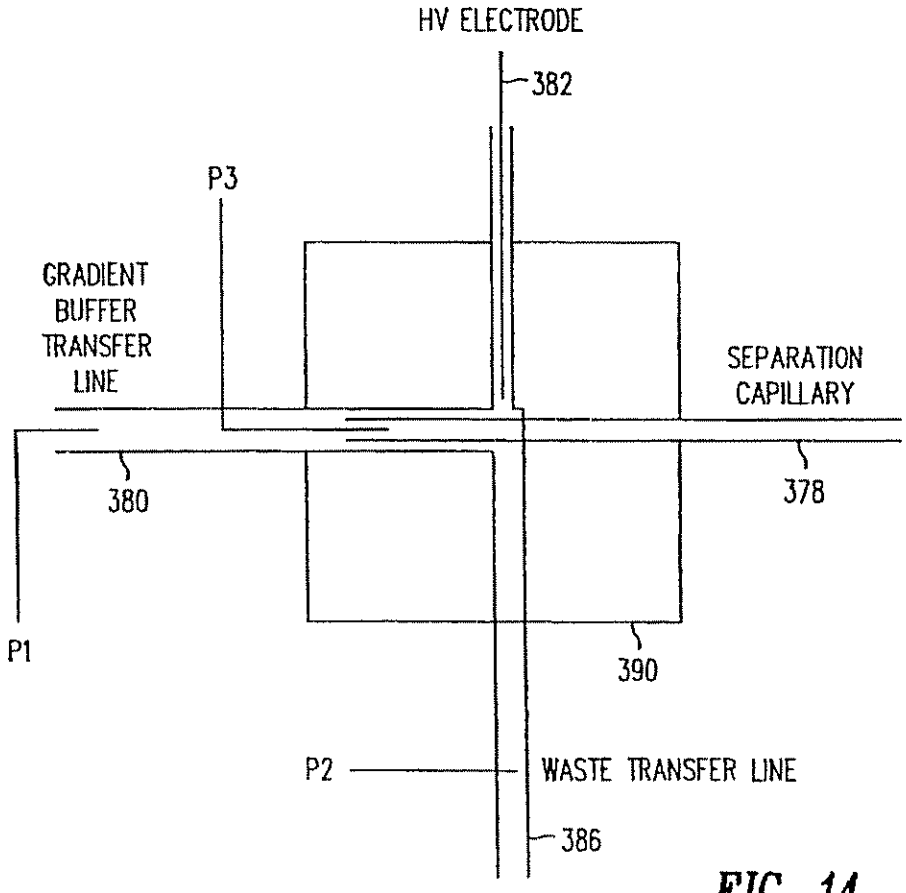
HV ELECTRODE
382
P3
GRADIENT BUFFER TRANSFER LINE
SEPARATION CAPILLARY
380
378
P1
390
P2
WASTE TRANSFER LINE
386

FIG. 14

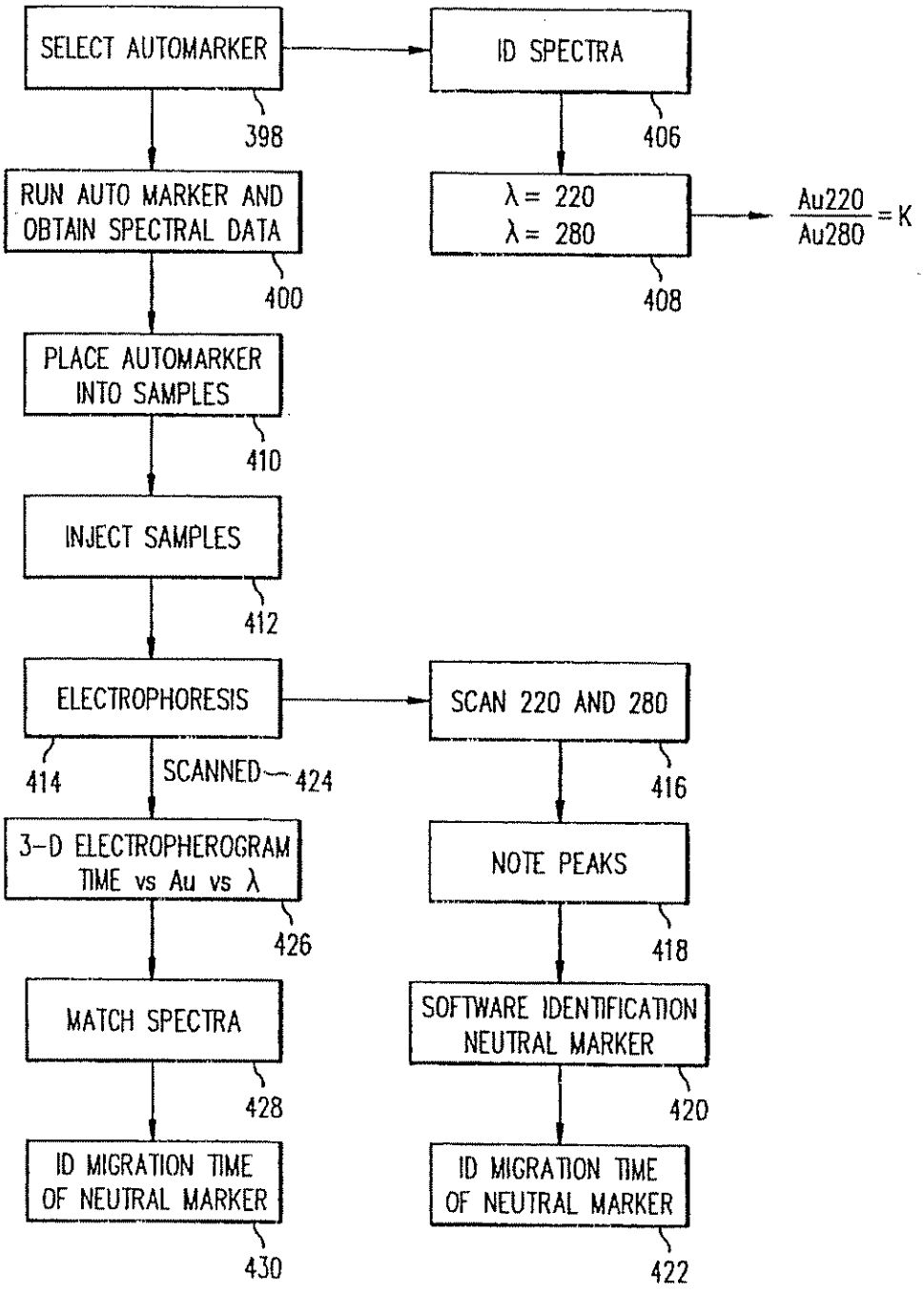
SELECT AUTOMARKER
398
RUN AUTO MARKER AND OBTAIN SPECTRAL DATA
400
PLACE AUTOMARKER INTO SAMPLES
410
INJECT SAMPLES
412
ELECTROPHORESIS
414
SCANNED
424
3-D ELECTROPHEROGRAM TIME vs Au vs λ
426
MATCH SPECTRA
428
ID MIGRATION TIME OF NEUTRAL MARKER
430
ID SPECTRA
406
λ = 220
λ = 280
408
Au220/Au280 = K
SCAN 220 AND 280
416
NOTE PEAKS
418
SOFTWARE IDENTIFICATION NEUTRAL MARKER
420
ID MIGRATION TIME OF NEUTRAL MARKER
422

FIG. 15

THERMAL CONTROL FOR CAPILLARY ELECTROPHORESIS APPARATUS

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to an apparatus and method for performing capillary electrophoresis, and more specifically to a thermal control system for a capillary electrophoresis instrument.

2. Description of the Prior Art

Capillary electrophoresis (CE) is a chemistry separation technique which utilizes the differences in solute electrophoretic velocity to isolate the various components of a sample. FIG. 1 depicts a typical CE apparatus. A high intensity electrical field supplied by high voltage power supply 10 is applied across a teflon, glass, or quartz separation capillary tube 12 of narrow inside diameter (5 to 400 micrometers) containing an electrolytic buffer solution. For an uncoated, open capillary tube, the presence of the electrical field imparts motion to charged and uncharged moieties present in the buffer through two mechanisms: electro-osmotic (endo-osmotic) flow and electrophoretic force. Flow of buffer (or sample from sample vial 14) through capillary 12 is detected by a detector 16.

Electro-osmotic flow is the bulk flow of buffer from a first buffer vial 18 to a second buffer vial 19 through capillary 12 due to the shearing movement of a diffuse layer of cations past a more firmly held, dense layer, interacting with integral, anionic groups of the capillary wall. Factors which influence the velocity of electro-osmotic flow are: electrical field strength; buffer dielectric constant; zeta potential (the electrical potential existing between diffuse and compact cationic layers); and buffer viscosity (which is dependent on bulk properties of the buffer and the temperature of the buffer). For electro-osmotically driven, packed capillary, reverse phase chromatography applications, solvents of use are any normally used solvent for standard reverse phase liquid chromatography.

Electrophoretic force is the force applied to charged particles residing in an electrical field, and neutral or uncharged molecules are not affected. Positively charged molecules (cations) migrate towards the cathode while negatively charged molecules (anions) move towards the anode. Factors controlling solute electrophoretic velocity are: molecular charge; electrical field strength; viscosity of the migration media; and solute molecular geometric factors.

The net velocity at which a solute travels in an uncoated, open capillary tube during CE is the vector sum of the electro-osmotic and electrophoretic velocities. Buffer viscosity plays a significant role for both of these phenomenon. Both electrophoretic and electro-osmotic velocities are inversely proportional to buffer viscosity, thus affecting the net migration velocity for all solutes.

When an electrical field is applied to a capillary which contains buffer, joule heating occurs. Accordingly the temperature of the buffer within the capillary increases until a steady state of heat exchange between the capillary and its surrounding environment is achieved. Consequently the ultimate buffer temperature is dependent upon the ambient temperature surrounding the capillary. Because of the temperature dependence of viscosity, the mobility of a solute in a given buffer within a given capillary in a given electrical field is largely determined by ambient temperature. For temperatures between 15° to 30° C., a 1° C. temperature increase results in an approximate 2 percent decrease in viscosity, increasing solute net velocity by 2 percent.

As is the case in many chromatographic techniques, solute identity is linked to migration time and velocity. For one form of CE known as capillary zone electrophoresis, samples are loaded into the capillary as a slug or plug. The latter may be achieved by application of an electrical field or some hydrodynamic force (vacuum or pressure head). An electrical field is then applied and the solutes migrate, as bands, down the capillary at their respective net velocities. Differences among these velocities create the primary mechanism for solute separation. These solute bands are then detected by monitoring a bulk property of the buffer such as refractive index, photometric absorbance, fluorescence, electrical conductivity, or thermal conductivity. The time period extending from the initiation of the separatory process to the point of solute detection is termed the migration time. The net velocity is determined using the migration time and the distance traveled by the solute.

Because of the high efficiencies achieved in capillary electrophoresis, it is not uncommon to see peak widths as narrow as two to three seconds. For complex solute matrices, multiple peaks may be separated by as little as two to three seconds in migration time. Consequently, a twenty minute CE run in which the temperature has changed by 0.1° C. can experience changes in migration time by as much as 2.4 seconds, possibly causing improper solute identification. Thus, efficient temperature regulation is required.

In the prior art, a capillary tube 12 as used in an electrophoresis instrument is supported in a variety of ways, depending on whether tube 12 is to be cooled by air, by liquid, or by metal plates in contact with the capillary tube. Cooling of tube 12 is important since the electrophoresis process subjects the capillary tube to a very high voltage which causes joule heating in the capillary tube. It is important to maintain the temperature of the tube at a stable predetermined temperature so as to be able to make measurements at a known temperature. Various schemes have been suggested for supporting and cooling the capillary tube, all of which have significant disadvantages and many of which are not suitable for air cooling purposes.

Prior art electrophoresis and similar spectrographic instruments typically include an optical path as shown in FIG. 2, which includes two light sources 22, 24 each of which provides a different spectra. Typically one light source 22 is a deuterium (D_2) source and the second light source 24 is a tungsten (W) light source. A movable shutter 26 is provided in front of light sources 22, 24 so as to switch in light source 22 or light source 24 depending on which spectra is desired. A light beam 28 from either light source is then passed through baffles 29 onto a concave holographic grating 30 or similar diffraction device, and then is focused into beam splitter 32 through baffles 33.

Beam splitter 32 in one form in the prior art is a short length of optical fibers. In the typical prior art instrument, a portion of the light transmitted to some of the optical fibers emerges from the beam splitter 32 at reference arm 34 and is sent via window 36 to a reference photodetector 38 which detects the reference light beam for purposes of comparison. The remainder of the light transmitted through beam splitter 32 is transmitted through a longer length of optical fibers to sample end

40 of the beam splitter and is focused using a lens 42 into sample cell 44 in which the sample is held. The portion of the light which passes through sample cell 44 and the sample therein is then directed onto a second (sample) photodetector 46 through window 48. The first and second photodetectors 38, 46 are matched substrate photodetectors, i.e. cut from the same piece of crystal or other photodetecting material, so as to have matching thermal properties. Also shown is monochromator casing 50. The dual beam approach compensates for fluctuations and the changes in intensity of the light source level, as well as any changes in intensity in the propagation of light.

For the purpose of remote detection in which only the sample arm is elongated, this prior art system has several disadvantages. Since reference photodetector 38 and sample photodetector 46 would be widely separated, they are subject to different amounts of heat due to their different locations. Thereby the problem of dark current i.e., drift caused by unequal heating, is significant, resulting in less precise measurements. Also, if the sample arm 40 of beam splitter 32 (i.e., that portion of the optical path which leads to the sample) is mechanically flexed, this flexing distorts the optical path through the optical fibers in sample arm 40, resulting in more or less light reaching sample cell 44. Since the portion of the light beam which reaches reference detector 38 is not so distorted, this causes a difference between the reference light beam and the sample light beam. Thereby, the prior art system is deficient because the common path of propagation is not maintained to the sample 46 and reference photodetectors 38.

Another significant problem with prior art electrophoresis instruments is the relative difficulty of controlling the temperature of the sample inside the capillary tube. As discussed above, capillary tubes are typically cooled by forced air or circulating liquid or by placing the capillary tube between metal radiator plates. The object is to cool and/or heat the capillary to a particular target temperature. Typically, the temperature control of the capillary tube in the prior art is performed by monitoring the temperature of the media surrounding the capillary tube. This process is problematic in that a thermal dam occurs at the interface between the media surrounding the capillary tube and the capillary tube itself. That is, thermal transfer is inhibited across this boundary, and therefore the temperature of the media surrounding the capillary tube is not exactly the same as that of the capillary tube itself.

As discussed above, electro-osmotic flow is the bulk flow of a solution to the capillary tube under high voltage which occurs in most forms of capillary electrophoresis in which the interior wall of the capillary tube has not been treated. It is well known that solutes move through the capillary tubing under the influence of the applied electric field at a net velocity equal to the vector sum of the electrophoretic velocity and the electro-osmotic velocity. Thus a cation, neglecting any solute-wall interactions, will have two mobilities or velocities in the same direction and thus will tend to move through the tubing relatively quickly. An anion will have an electrophoretic velocity which is the vector opposite direction of the electro-osmotic velocity and thus will tend to move through the capillary tubing relatively slowly. A non-charged species i.e., a neutral species, will have no electrophoretic velocity at all and thus can be used to measure the electro-osmotic velocity of the system. Typically amides or some other neutral species are used to measure electro-osmotic velocity. These materials are typically known as neutral markers. The term neutral marker refers to the fact that in the buffer of interest, the neutral marker solute has no electrical charge.

In the prior art, electro-osmotic flow is determined by introduction of a neutral marker and then observing at one particular wavelength the flow of the neutral marker through the system to identify when the neutral marker passes the detector. This process works well with very simple sample combinations, where no other solutes co-migrate with the neutral marker. If however other compounds present in the sample combination are also neutral, this complicates the process of detecting the neutral marker.

It is also known to detect electro-osmotic flow without the use of a neutral marker. In one known process, the electro-osmotic flow is determined by the level of current stabilization when different buffer solutions having different specific conductivities were provided in the anode and buffer reservoirs. This process relies on the assumption that the system demonstrates a zeta potential and dielectric constant which is not seriously affected by the change in the electrolyte composition in the solutes. In another method, electro-osmotic flow is determined without the use of a neutral marker by observing continuously the weight of the material held in the cathode buffer reservoir. The volume transfer is then determined by dividing the change in mass of the cathode buffer reservoir by the density of the buffer. These last two methods are extremely time consuming and difficult and require significant manual intervention in addition to being of doubtful accuracy. Thus, there is a significant need for a method to determine the electro-osmotic flow by an automated process which can deal with complex sample combinations.

SUMMARY OF THE INVENTION

In accordance with the invention, various improvements are made to an electrophoresis instrument for purposes of improving the accuracy and usability of the instrument and to allow measurements not obtainable using the prior art instruments.

In accordance with the invention, capillary tubing is coiled and enclosed in an air cooled cartridge. The air cooled cartridge includes a housing, electrodes fitted to the capillary tubing, and a spherical lens assembly which is part of the optical path. The air cooled cartridge holds the capillary tubing so as to optimize air cooling of the capillary tubing when the cartridge is installed in the instrument. The air cooled cartridge fits into a manifold which includes both an anode and a cathode subassembly for holding vials containing the sample or buffer solutions and a ground potential chamber.

Also provided in accordance with the invention is a method of marking the air cooled cartridges using a bar code so as to provide identifying information for automated handling of the cartridges.

Also in accordance with the invention, a remote optical path is provided in which a fiber optic bundle having a particular arrangement of optical fibers for carrying the sample and reference light beams has an extended reference arm for carrying the reference light beam to the reference detector, which is located in close proximity to the sample photodetector. Thus the reference photodetector is in the same environment, i.e., heat level, as is the sample detector. This structure is advan-

tageous in that remote transference of detection light in the common arm of the bifurcated optical fiber bundle reduces the optical system sensitivity to mechanical perturbations to the optical fibers. Thus slight changes are viewed simultaneously by both the sample and reference photodetectors and are thus more easily corrected.

In accordance with another aspect of the invention, the temperature of the capillary tubing during electrophoresis is controlled by observation of the electrical resistance of the capillary tubing. This method relies on the determination that the electrical resistance of the tubing containing a given buffer is a unique function of its temperature. Thus resistance may be calculated from the observed voltage and current across the capillary, and the capillary tubing may be air cooled by provision of an air flow across the capillary tubing in response to the observed resistance.

The provision in accordance with the invention of very precise reproducible temperature control provides the ability to perform thermal gradient electrophoresis in the instrument. It has not been possible previously to perform this process in a reliable, reproducible manner since the required temperature control equipment was not in existence.

Also in accordance with the invention, a method is provided of determining electro-osmotic flow by use of a neutral marker in which the spectral characteristics of the neutral marker are identified and used to determine when the neutral marker has passed the detector. The method of observation and determination of the spectrum associated with the neutral marker allows use of determination of electro-osmotic flow even in the case of co-elution or co-migration of a solute which is similar in its electro-phoretical profile to that of the neutral marker.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** shows a prior art electrophoresis apparatus.

FIG. **2** shows a prior art optical path for the UV-visible detector which may be used in the device of FIG. **1**.

FIGS. **3**(*a*) to **3**(*c*) show views of an electrophoresis instrument in accordance with the invention.

FIG. **3**(*d*) shows schematically a column conditioning and hydrodynamic injection system in accordance with the invention.

FIG. **4** shows an air cooled cartridge in accordance with the invention.

FIG. **5** shows the air cooled cartridge partially inserted into the temperature control system.

FIGS. **6** and **7** show a temperature control system in accordance with the invention.

FIG. **8** shows a manifold in accordance with the invention.

FIGS. **9**(*a*) to **9**(*f*) show a remote optical path in accordance with the invention.

FIG. **10** shows detail of the fiber optic bundle used in the remote optical path.

FIG. **11** is a flow chart showing a temperature control method in accordance with the invention.

FIG. **12** shows a calibration plot for temperature control.

FIG. **13** shows a gradient micellular electrophoresis apparatus in accordance with the invention.

FIG. **14** shows detail of the gradient micellular electrophoresis apparatus.

FIG. **15** is a flow chart showing use of a neutral marker in accordance with the invention.

Similar reference numbers in various figures denote similar or identical structures.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the invention, various improvements are provided over the prior art electrophoresis apparatus.

FIG. **3**(*a*) shows a front view of an electrophoresis instrument in accordance with the invention. Shown in enclosure **56** is the front panel **58** in a closed position, air cooled cartridge loading port **60**, information displays **62**, **64**, and control buttons **66**.

FIG. **3**(*b*) shows a rear view of the instrument of FIG. **3**(*a*). Shown in enclosure **56** are high voltage power supply **70**, high voltage line **72**, power transformer **74**, vacuum exhaust port **76**, oven purge port **78**, and helium inlet port **80**. The rear of the detector **82** portion of the instrument is shown. Also included are RS232 connector **86**, I/O port **88**, power switch **90**, and voltage selector and fuse block **92**.

FIG. **3**(*c*) shows a front view of the instrument of FIG. **3**(*a*), with the front panel removed. Shown are electric power board **98**, a conventional autosampler **100**, buffer solution bottle **102**, helium valve **104**, dessicant bottle **106**, waste trap **108**, injection vacuum tank **110**, pressure transducer **112**, vacuum pump **114**, fluid pump **116**, valve **118**, beam splitter **120**, optical bench **122**, manifold **124**, oven (thermal chamber) **126**, fan **128**, and air cooled cartridge **130**.

FIG. **3**(*d*) shows schematically a column conditioning and hydrodynamic injection system for the above described instrument in accordance with the invention. Shown are fluid pump **116**, vacuum pump **114**, valve VA, valve VB, valve VD, cathode reservoir **136**, vent line **138**, valve VE, valve VF, valve VC, atmospheric pressure line **140**, injection vacuum chamber **110**, pressure transducer **112**, and control microprocessor **142**.

Air Cooled Cartridge

An air cooled cartridge **130** (see FIG. **4**) is used for capillary electrophoresis in accordance with the invention. Cartridge **130** consists of: a main body **146**; a bobbin assembly **148**; a spherical lens holder assembly **150**; metallic electrodes **152**, **154**; electrical contacts **156**, **158**; and capillary tubing **162** of glass, quartz, or teflon, typically no greater than 500 microns in inside diameter and about 10 to 200 cm. long.

Main body **146** is a support for the other subassemblies. Additionally, main body **146** aligns the electrode **152**, **154** and optical **150** subassemblies with their respective counterparts in the manifold and remote optical path (not shown here but described below).

Bobbin assembly **148** supports capillary **162** which is coiled in a concentric circle. Bobbin **148** consists of a central support ring **166** with radiating capillary support pieces **168**, **170**, etc. Each capillary support piece **168**, **170**, etc., contains four equally spaced holes (not shown) and one hole centered above the array of four, through which capillary **162** is threaded and held in place. The thickness of each support piece **168**, **170** is minimized, maximizing the capillary surface area exposed to ambient air.

Spherical lens holder **150** fastens capillary **162** to the cartridge main body **130** prior to entry of capillary **162** to electrode **152** as well as holding capillary **162** in the proper orientation with spherical lens holder **150**, permitting precise image focusing into the capillary lumen,

thus limiting stray light. Spherical lens holder **150** mates with the remainder of the remote optical path (not shown) to provide precise, reproducible optical alignment, as described below.

Metallic electrodes **152, 154** are constructed of high conductivity, low electrochemical reactivity metals. An alloy of platinum-iridium is used in one embodiment. A portion of capillary **162** exits cartridge **130** and enters the manifold (not shown) by passing through the center of electrodes **152, 154**. Electrodes **152, 154** each have an inside diameter slightly larger than that of the outside diameter of capillary **162**. This minimizes the dead volume between electrodes **152, 154** and capillary **162**.

Air cooled cartridge **130** is a structure approximately five inches (12 cm.) wide, nine inches (22 cm.) high, and 0.25 inch (0.5 cm.) thick. Main body **146** of the air cooled cartridge is preferably molded from black delrin. Other low thermal mass, low thermal conductivity, and high dielectric strength materials may be used. The dimensions of air cooled cartridge **130** may be otherwise as convenient. Air cooling slots **171-1, 171-2, . . . , 171-n** are formed in main body **146**. Spherical lens holder **150** is preferably made of black UV stabilized ABS and is a flange-like structure with a smaller portion which fits inside a cavity provided in main body **146** and with a lip for fitting against main body **146** to fix lens holder **150** in place. Other high dielectric strength, UV stable materials may also be used for lens holder **150**.

Bobbin assembly **148**, around which capillary tube **162** is concentrically wound, is formed of delrin and is about 3.5 inches (9.0 cm.) in diameter and fits inside a cavity provided in main body **146**.

As shown in FIG. **5**, air cooled cartridge **130** is partially lowered into a chamber **174** at one side of which is provided a Peltier type heat sink device **176**, which is a well known type of solid state device for cooling and/or heating to precise temperatures. On each side of cartridge **130** is an insulative layer of polyethylene **178, 180**, each layer **178, 180** approximately 0.78 inches (2.0 cm.) thick. Air cooled cartridge **130** when fully lowered into position between insulative layers **178, 180** is locked in place by a cartridge lock bar **182**. A retaining thumb screw **184** is also provided. Also provided is a fan (the blades of which are hidden and not shown) mounted on panel **187** driven by a regulated DC motor **188** fitted with a heat sink assembly **186** for drawing the air cooled by Peltier heat sink **176** across the capillary (not shown) in cartridge **130**.

The cooling system in accordance with the invention is shown schematically in FIG. **6** showing DC motor **188** for driving fan blades **189**, and also installed on panel **187** supporting fan blades **189** is a temperature sensing, resistive thermal device **192** (RTD). As shown, fan blades **189** draw the air (shown by lines) through the center of bobbin **148**. The air is then recirculated by fan blades **189** across capillary **162** to the temperature regulating heat exchange surface **197** of the Peltier device.

Peltier device **176** is sandwiched between conventional heat exchanging surface **197** and conventional heat dissipating/collecting surface **198**. Surfaces **197, 198** are separated by a 0.25" (6.3 mm) thick layer of polyethylene insulation **199**.

As shown in FIG. **7** in a block diagram, RTD device **192** provides a measurement of the temperature of air surrounding capillary **162**. The capillary electrical resistance is determined by dividing the applied voltage (usually about 5 KV) by the measured current during a calibration phase. This resistance information is provided to microprocessor **142** which is part of the electrophoresis instrument and which in one embodiment is a Motorola 68008 microprocessor. This microprocessor then uses the ambient air temperature and capillary resistance data to control the drive current **200** to Peltier device **176** so as to maintain a constant electrical resistance and set point temperature **202** in capillary **162** during the separation process. The actual set point is the capillary resistance. Ambient temperature (not shown) is used as a secondary parameter to anticipate the arrival at the desired capillary resistance, thus minimizing set point setting time.

The lower portion of the air cooled cartridge when in a lowered position is in contact with a manifold **124**, as shown in FIG. **8**. The air cooled cartridge (not shown) fits into alignment slots **210, 212**. Manifold **124** includes a high potential (anode or cathode) subassembly, including high voltage contact **214**, which accepts vials containing either sample solution or buffer solution and also includes a central support subassembly **220**, and a ground potential chamber **224**, containing high voltage contact **216**, which is connected to a valve assembly via port **226** which allows (under automatic control) the filling and flushing with buffer and application of vacuum to the capillary tubing in the air cooled cartridge for the purpose of rinsing, washing, or hydrodynamic injection. These processes are performed by the structure shown schematically in FIG. **3**(*d*). Also provided in manifold **124** is a hole **230** for the optical bench (not shown, described below) to slide into so as to contact the spherical lens assembly (not shown, in the air cooled cartridge). A vial-like chamber **232** is built into the manifold structure so as to eliminate the need for a ground potential buffer vial. Also provided are high voltage line entrance **236** and high potential vial holder **238**.

Bar Code on Cartridge

In accordance with the invention as shown in FIG. **4**, air cooled cartridge **130** is marked with a bar code index **246** at a convenient location to identify the particular cassette. Also, additional information is included in bar code index **246**. This information includes the length of capillary tube **162** in that particular air cooled cassette. The length of the tube is required as described above for determining electrical field strength, and electroosmotic and electrophoretic mobilities and velocities. The length is also required to calculate the fluid-flow resistance in the capillary tube. The fluid-flow resistance is necessary for the system to determine automatically how long it takes for the capillary tube to be flushed with a given solution and what would be the approximate volume of sample loaded into the capillary tube for a given vacuum applied for a given period.

The system is automatically informed by reading bar code index **246** of the inside diameter of capillary tube **162**. This is necessary for determining the fluid resistance of the capillary tube and the electrical resistance of the capillary tube for the above stated reasons.

The system is also informed automatically by bar code index **246** whether the tube is an open capillary tube, i.e. contains no gel, or is a closed tube, i.e. contains gel. This is important for hydrodynamic or vacuum type injections because the gel would be damaged or destroyed by application of a vacuum or hydrodynamic forces. Bar code index **246** also indicates whether the tube has its interior lined with a coating for purposes of

knowing whether there is significant electro-osmotic flow in the capillary tube.

Also, as described above, each particular cartridge 130 is identified with its own particular number in bar code index 246 so that the system can automatically track the performance of each cartridge and/or capillary tube based on the separation efficiency for a given test.

Bar code index 246 on air cooled cartridge 130 is read in one embodiment of the invention by a conventional bar code reader (not shown) which is part of the electrophoresis instrument. Thus the bar code reader in the instrument reads bar code index 246 on a particular air cooled cartridge and provides the information in the index to the microprocessor and related computer software for the above described purposes.

Remote Optical Path

The remote optical path as shown schematically in FIG. 9(*a*) includes a unique fiber optic beam splitter 120 (as in FIG. 3(*c*)) arrangement for detecting small sample volumes (down to about 100 picoliters) in a capillary. Light 260 is focused into a fiber optic bundle 262 from the exit slit of a conventional monochromator (including one or two light sources, a shutter, and a diffraction grating as in FIG. 1 and not shown here). The monochromator may be generating light of a given bandwidth for the purpose of UV-visible photometric absorbance detection, fluorescence detection, refractive index detection, as well as any other means of photometric detection. Light may also be focused into fiber optic bundle 262 from a coherent light source (laser) for the purpose of refractive index, fluorescence, thermal-optical-density detection, as well as other means of coherent light photometric detection. Fiber optic bundle 262 is remotely bifurcated at point 264 into a sample arm 266 and a reference arm 268. The light exiting from reference arm 268 impinges onto a reference photodetection device 272 located in the same environment as the sample photodetection device 274. Light emitting from sample arm 266 is focused using a plano-convex lens 276 into a second, spherical lens 280 in direct contact with the capillary 162.

The fibers of fiber optic sample arm 266 may be arranged in cross section in a circle, rectangle, square, trapezoid, or other parallelogram or triangular pattern in order to facilitate the focusing of the image into the center of the capillary. Sample photodetector 274 is placed directly behind capillary 162. The beam splitter housing self-aligns via locating hole 230 in the manifold 124 (see FIG. 8) and on the cartridge lens holder 150 (see FIG. 4). Spherical lens 280 is thus located in the cartridge lens holder 150, while the plano-convex lens 276, reference photodetector 272, and both ends of the fiber optic bundle 262 are housed in a retractable member (not shown) which slides into and out of the spherical lens holder 150 which is mounted on cartridge 130 (see FIG. 4).

This structure is advantageous in that remote transference of detection light in the common arm of a bifurcating fiber optic bundle greatly reduces the optical system's sensitivity to mechanical perturbations to the fiber optics. In this approach, light changes are simultaneously viewed by sample 274 and reference photodetectors 272 and are thus correctable.

Placement of both photodetectors 272, 274 in a similar environment reduces perturbations resulting from physico-mechanical variances in detection environments. The combination of lenses produces an image of appropriate size for small volume detection without any attendant loss of throughput. The mechanical layout of the system is such so that all optical elements are self-aligning.

FIG. 9(*b*) shows detail of the optical path at its upper end down to the bifurcation point. Shown are beam splitter body 284, an insert 286 in body 284 to hold the optical fiber bundle 262, the optical fiber exterior PVC monocoil coating 288, PVC shrink tubing 290 over the optical fiber bundle, and a dual plug body 292. Optical fiber bundle 262 bifurcates into a reference arm connector 296 and sample arm connector 298, both connected mechanically by a connector 300. Connector 300 is shown in a side view in FIG. 9(*c*). A spacing "d" of about 1.094" (28 mm) is provided between the center of the reference arm 268 and sample arm 266. The short axis of the sample fiber bundle is parallel to the long axis of the capillary and perpendicular to a horizontal line defined between the center points of the sample and reference arms.

The above described structure is fastened together with 2039 type epoxy. A 360° twist is provided in the fibers in the common sector 288 to increase flexibility.

In accordance with the invention the cross-section shape of the sample fiber optic bundle 266 may be varied in accordance with the application. For instance, in the case where the light beam in the sample arm is to be transversely focused into a cylinder such as the capillary, it is most desirable to provide a rectangular cross-sectional shape light beam. Thereby the fiber optic bundle is provided in a rectangular or parallelogram shape. In another case when it is desirable to focus the sample light beam into a cylindrical flow cell as in a liquid chromatography detector, then it is desirable to have a circular shape of the cross-section of the light beam and thereby the fiber optics are bundled into a circle in cross-section.

FIG. 9(*d*) shows the optical fiber pattern in sample arm 266 in a rectangular cross sectional arrangement. The overall width w is about 3.05 mm; the height h is about 0.46 mm. Shown are optical fibers a-1, a-2, . . . , a-24.

FIG. 9(*e*) shows the lower end of the remote optical path, with the beam splitter common trunk 262 bifurcating into the sample arm 266 and reference arm 268, both entering beam splitter block 304. Each arm 266, 268 is respectively attached to block 304 by a set screw 306, 308. The reference photodiode assembly 272 is shown, as is lens shroud 310 to carry the sample light beam to the sample cell (not shown). Beam splitter block 304 is fastened to platform 314, which is attached to support 316 by a set screw 320 and a set of dowels 321 (only one shown).

Detail of the sample photodiode assembly is shown in FIG. 9(*f*). Shown are the photodiode housing 322, spring assembly outer ring 324, and spring assembly inner ring 326.

FIG. 10 shows an end-on view of fiber optic bundle 262 showing that the fiber optic bundle 262 is composed of a number of triads of single optic fibers. Each triad consists of one reference type fiber 330 (shown by shading) and two sample type fibers 332, 334 (shown in white). The fibers themselves are identical between the sample and reference fibers. The designation of reference or sample merely indicates to which photodetector the optic fiber directs its light. The fiber triads are arranged in conjunction with each other so as when one

moves from one reference plane at the entry portion of the beam splitter to another reference plane the triad is always conserved, so that at any angle the light is introduced to two sample and one reference optic fibers. Fiber optic bundle 262 in total includes in one embodiment 37 optic fibers. The diameter of common fiber optic bundle 262 is preferably about 0.067 inches (1.7 mm). This is a matter of design choice, and is not limiting in accordance with the invention. Twice as many sample fibers are provided as reference fibers, since the sample light beam must pass through the capillary tubing and other optics and thus there is more loss of throughput in the sample light beam.

In one embodiment of the invention, the fiber optic bundle is custom made. The optic fibers are ultraviolet transparent quartz approximately 200 microns in diameter, with a 20 micron thick cladding, and a 12.5 micron thick polyimid coating. The optical fibers are 200/220/245 superguide G type. The bundle is supported loosely by a 0.125 inch (3.1 mm) inside diameter teflon tube in a PVC monocoil outer jacket. The fiber optic bundle in one embodiment is provided by Highlight Fiber Optics in Caldwell, Id. The approximate overall length of the beam splitter is 28 inches (70 cm.). The point of bifurcation between the sample arm and the reference arm is at 26 inches (65 cm.) from the entry portion of the beam splitter.

Constant Capillary Electrical Resistance Temperature Control

Also in accordance with the invention, constant resistance cooling of the capillary is provided. As described above, the electrical resistance of the capillary provides a means of sensing the temperature of the capillary. Therefore, a method is provided for measuring and controlling the temperature of the capillary using the apparatus as shown in FIG. 6.

It is well known that the electrical resistance of the capillary is directly proportional to the capillary length and inversely proportional to the capillary radius squared. The solution electrical resistance is inversely proportional to the temperature of the solution and is inversely related to the specific conductivity of the solution in the capillary. This means that for a capillary of a given size and a given length containing a given solution, the electrical resistance is a direct function of the capillary temperature. In accordance with the invention, the high voltage power supply's current and voltage conventional sense lines are used to measure the electrical resistance of the capillary, and so in effect the capillary is used as a thermometer.

A control procedure is provided to control the temperature of the capillary. This control procedure is a control program associated with the above-mentioned microprocessor 142 (see FIG. 7) resident in the electrophoresis instrument. The procedure for temperature control is shown in a flow chart in FIG. 11.

In accordance with the invention, the following steps are used in order to control temperature. First, a voltage start slope is selected at step 340. (See voltage vs. time plot, FIG. 12.) This is the rate (shown by the dotted line in FIG. 12) at which the ultimate separation voltage will be applied. For example, if the electrophoresis separation voltage of 30 KV is achieved in 10 seconds, then the start slope is 3 KV/second. Second, a set point ambient temperature is selected at step 342 for the capillary temperature as desired. This is done by the conventional method of monitoring the temperature of the air around the capillary tube and allowing sufficient time at step 344 for the heat transfer process to take place until the capillary tube approaches the target temperature and therefore the temperature in the capillary is very close to that of the surrounding air.

In the next step 346, the electrophoresis separation process in the capillary begins by performing a sample injection and beginning the run by increasing at step 348 both the current and the voltage of the electric power provided to the capillary. During the calibration phase the current and the voltage are increased at a particular steady rate, equivalent to two times the start slope at step 350. Capillary resistance is calculated during the hold time at step 352 (shown as about 0.8 to 2.4 seconds in FIG. 12) at 5 KV, at which level typically there is no significant joule heating. In the next step 354 the weighted average resistance or average resistance for the calibration period hold time is calculated. This calculated resistance is then attributed to the resistance of the system at the selected set point temperature. The voltage level is increased to 10 KV at twice the selected start slope in step 356. Then the voltage is further increased to the set voltage at the selected start slope in step 358.

The next phase in steps 360 to 362 is the temperature control phase. The resistance is monitored at step 360 at a particular duty cycle, i.e., for instance 50 times per second, by measuring the capillary current and voltage, and then in step 362 heat is either pumped into or out of the chamber in which the air cooled cartridge is housed by use of the previously described fan and Peltier device. Thus the electrical resistance of the capillary is maintained at a constant level, providing a constant temperature.

Buffer Gradient And Temperature Gradient Capillary Electrophoresis

Micellular electrophoretic chromatography is known in the art. (See Terabe, *J. of Microcolumn Techniques*, Vol 1, No. 3, 1989, p. 150.) This technique involves formation of a micell in the sample by providing a buffer solution containing amphophilic complexes which bind by non-polar or lipophillic attraction. They remain soluble in aqueous environments due to their polar moieties. For capillary, micellular electrokinetic chromatography, typically buffer solutions composed of acid or base salts (including but not limited to phosphate, tris, hepes, citrate, borate, amino acids, and other zwitter ionic buffers) in concentrations from 0.01 millimole to 500 millimole are used in conjunction with a detergent or other lipid-like moiety which forms micells. The micell producing agent (including but not limited to sodium dodecylsulfate, bile acids, etc.) is added until reaching minimal micell concentration for the given temperature.

In accordance with the invention, micellular, open tube separations take advantage of the differences in the partition coefficients of various solutes so that the higher the partition coefficient the longer the solutes stay in contact with the micell under the influence of the electric field in the electrophoresis instrument. Thus it is possible to separate neutral compounds on the basis of their partition coefficients. However, a problem arises in trying to separate solutes of similar partition coefficients or whose partition coefficients are so large that they co-migrate on the micells and are never separated. In buffer gradient electrophoresis, the buffer composition is changed over time and thus because the

basic function of the partition coefficient is dependent on the two phases, polar and non-polar components (polar component being the buffer and the non-polar, the micell), the solubility of the solute in the buffer is changed. Thus as the lipophilicity of the buffer is increased, those compounds that have slightly lower partition coefficients will come off the micell. Thus the compounds are selectively removed from the micell as a function of time and thus contact the detector in the instrument and are observed.

The gradient micellular chromatography apparatus is depicted in FIG. 13. A pair of conventional microliter syringe pumps 370, 372 are driven at different rates to displace different amounts of fluids which when mixed comprise the buffer. Mixing occurs in a conventional micromixer 376 and the resultant mixture is transported to separation capillary 378 via gradient buffer transfer line 380. High voltage electrode 382 creates an electric field in separation capillary 378.

Fluid from the gradient buffer transfer line 380 enters separation capillary 378 (see FIG. 14 showing detail of the device of FIG. 13) via electro-osmotic flow (and not parabolic pressure driven flow) as long as the pressure head at point P3 is much greater than that at point P2. The excess buffer exits via waste transfer line 386. Sufficient mixing response time is achieved using this split-flow approach.

The microliter pumps 370, 372, micromixer 376 and separation capillary 378 are either held at ground potential or enclosed in a Farraday cage 390 to protect against electrical shock.

In accordance with the invention, gradient micell electrophoresis may also be achieved by temperature programming. The Gibbs free energy of binding between the solute and micell is determined by the sum of the binding enthalpy and the temperature-entropy product ($\Delta G^{\circ}=\Delta H^{\circ}-T\Delta S^{\circ}$). If ΔG° is negative, binding occurs. In thermal gradient micellular capillary electrophoresis, temperature is increased as a function of time. Consequently, the temperature-entropy product also increases. When the temperature-entropy product exceeds the enthalpy of solute-micell binding, the solute is released from the micell and thus migrates at a faster rate to the detector. In order for this process to be used in a reliable, reproducible manner, precise temperature control is required. Such control is possible using the previously described constant resistance cooling technique.

Electro-Osmotic Flow Using Automatic Neutral Marker

In accordance with the invention, a method is implemented by use of the control program resident in the instrument's computer software for identification of neutral markers. This process is shown in flow chart form in FIG. 15.

In the first step 398, the user of the instrument selects a particular neutral marker substance. The neutral marker is selected as having a known spectrum and preferably having a spectrum greatly different from those of the solute molecules of interest. The selected neutral marker is then injected into the system in step 400 as a single component separation and its spectral characteristics measured. In the case of particular auto markers such as, for example, tryptophan at its pI (isoelectric pH), it is known that this auto marker will have an absorbance maximum at approximately 220 nanometers wavelength and a second absorbance maximum at approximately 280 nanometers wavelength as in step 406. Thus the system, based on data provided to it, will use the ratio of the absorbance at 220 nanometers to the absorbance at 280 nanometers to provide an identifying value at step 408 for this particular neutral marker. The selected neutral marker is then added in the appropriate concentration to the sample in step 410. A typical concentration is 0.1 milligram per milliliter. The samples containing the added-in neutral markers are then injected in step 412 into the capillary in the system.

In the next step 414, electrophoresis is conventionally performed. In accordance with one embodiment of the invention in steps 416 to 422, the spectrophotometric scanning is performed at step 416 at the two wavelengths of interest, 220 and 280 nanometers. In the next step 418 all peaks at these wavelengths are identified. Since other materials in the sample may also give peaks at 220 and 280 nanometers, the ratio of the absorbance at these two wavelengths is used to particularly identify at step 420 by a computer program the particular neutral marker tryptophan selected in the first step 422. Thus when this particular ratio is detected by the detector in the electrophoresis instrument, this identifies at step 422 the migration time of the neutral marker from the point of sample injection to the detector in the system.

In another embodiment of the invention in steps 424 to 430 the entire spectrum is scanned at a number of wavelengths at step 424. Then the system, by means of computer software, constructs at step 426 a three dimensional electropherogram of time versus absorbance versus wavelength. This electropherogram is then sliced perpendicular to the temporal, i.e. time axis, and then flipped around. This produces a spectrum of absorbance versus wavelength. This method allows identification of the spectrum associated with a particular neutral marker selected to provide a distinct spectrum. Thus when this particular spectrum is detected by the instrument in step 428, the time of detection determines the migration time of that particular neutral marker from the point of injection to the point of detection in the system in step 430. This method of scanning all wavelengths is more precise than the two wavelength method of steps 416 to 422 because it provides a better means of eliminating the problem of co-elution or comigration of solutes which are similar in their electrophoretic profiles to that of the neutral marker.

The velocity of electro-osmotic flow for both embodiments associated with the system is then determined by using the above-determined data from the neutral marker in steps 422 or 430. This determination is made in a post-run integration process (not shown). It is well known that electro-osmotic mobility is electro-osmotic velocity divided by electric field strength. Field strength is defined as voltage per column length. The velocity is the distance traveled from the beginning of the capillary at the point of injection to the point of detection of the neutral marker divided by the time required for this movement. Length L is the total length of the column from beginning to end and voltage is the applied voltage. Thus use of the neutral marker in detection hereof as described above allows calculation of the electro-osmotic velocity and of the electro-osmotic mobility.

The above description of the invention is illustrative and not limiting. Further modifications will be apparent to one of ordinary skill in the art in light of the disclosure and the appended claims.

We claim:

1. A system for controlling temperature in an instrument including a capillary tube holding a sample comprising:

air circulating means for circulating air across the capillary tube;

temperature sensing means for sensing the temperature of the air surrounding the capillary;

resistance sensing means for sensing an electrical resistance of the capillary tube;

heat means for providing heat to and removing heat from the air circulating across the capillary tube; and

control means for receiving the resistance sensed by the resistance sensing means and the temperature of the air surrounding the capillary sensed by the temperature sensing means and controlling the heat means so as to provide more or less heat to maintain the capillary tube at a predetermined temperature.

2. The device of claim 1, wherein the heat means includes a Peltier device.

3. A method for controlling temperature of a capillary tube comprising the steps of:

determining a target temperature for the capillary tube;

applying a voltage to the capillary tube;

observing the electrical resistance of the capillary tube as a result of the applied voltage;

determining the present temperature of the capillary tube as a function of the observed electrical resistance; and

controlling the temperature of a flow of air provided to the capillary tube from the present temperature so as to warm or cool the tube to the target temperature.

4. The method of claim 3, further comprising the step of providing an increase in voltage to the capillary tube and observing the electrical resistance of the capillary tube as a function of the change in voltage over time.

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